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Ter Mutation and Susceptibility to ΦX174 Phage in *E. coli* K12

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SUMMARY : The Ter-15 mutant derived from *E. coli* K12 W2252-11U⁻ RC^{str} (wild type I) is found to be sensitive to φx174 phage infection. Lipopolysaccharide extracted from this mutant inactivates the phage, and has core oligosaccharides identical in amounts to those in the lipopolysaccharide from wild type cells.

In contrast, the Ter-21 mutant derived from *E. coli* K12 W2252-11U⁻ RC^{rel} (wild type II) is not sensitive to this phage infection, and its lipopolysaccharide does not inactivate the phage. Its lipopolysaccharide sugars are found to be D-glucose and D-ribose, thus differing from the lipopolysaccharide sugars of the wild type cells.

ΦX174 phage contains a single strand circular DNA (ss-DNA) of 5375 nucleotides (1) in an icosa hedral coat with spikes at the 12 particles (2). From the reports of Lindberg (3) and Jazwinski et al (4), ΦX174 phage is able to infect the rough strain, but cannot infect the smooth or semirough strains. The susceptibility of the *Escherichia coli* strain to ΦX174 phage infection is primarily dependent upon whether or not the phage can bind to specific receptor attachment sites on the cells. With regard to ΦX174 phage binding, it is found the isolated cell wall inactivates the phage (5) and releases the DNA (6). Further, the purified lipopolysaccharides (LPS) derived from sensitive strains are demonstrated to be the receptors for this phage (7,8). Also, the structure of the polysaccharide chain in the LPS is identified as crucially important for receptor activity (3). Jazwinski, Lindberg and Kornberg (4) demonstrated that the ΦX174 and the related phage S13 receptor is found in the LPS of the cell outer membrane of sensitive strains of *E. coli* and *S. typhimurium*, and showed this phage binding site to be represented by the structure found in the core and backbone of the LPS.

Abbreviations used : LPS, lipopolysaccharide ; ss-DNA, single strand circular deoxyribonucleic acid ; dTDPG, thymidine 5'-diphosphate glucose ; KDO, 2-keto-3-deoxymannulosoctonic acid.

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We are interested in the change that occurs in ϕx174 phage infection in resistant and sensitive strains through decreased concentration of thymidine 5'-diphosphate glucose (dTDPG) which is formed in growing cells as a precursor for synthesis of LPS of the cell's outer membrane. *Escherichia coli* K12 W2252-11U⁻ (wild type I) is not sensitive to ϕx174 phage, but its Ter-15 mutant (9) becomes sensitive. Neither *Escherichia coli* K12 W2252-11U⁻ (wild type II)(non-isogenic strain to type I) nor its Ter-21 mutant strains is sensitive to ϕx174 phage. To resolve the discrepancy in the Ter-mutation from *E. coli* K12 strains, the LPS were extracted from the wild types and their Ter mutant strains, and their sugar contents were analyzed in detail by thin-layer and gas-liquid chromatography.

In this paper, we describe the phage sensitivity, the phage inactivation by the LPS from these *E. coli* cells, and the sugar components of the LPS.

Materials and Methods

Bacteria : *Escherichia coli* K12 W2252-11U⁻ (thy⁻,ura⁻,met⁻) RC^{str} (wild type I) and its dTDPG pyrophosphorylase deficient mutant is named Ter-15 (9). *Escherichia coli* K12 W2252-11U⁻ (thy⁻,ura⁻,met⁻) RC^{rel} (wild type II) and its dTDPG pyrophosphorylase deficient mutant is named Ter-21 (9). *Escherichia coli* C strain is used as an indicator for ϕx174 phage infection.

Phage : ΦX174 phage, wild type.

Chemicals : Uracil (Ura) and thymine (Thy) were purchased from PL-Biochemical Company, LTD. Casamino acid was from Difco laboratories, Detroit, U.S.A. Methionine, hexose and pentose and other chemicals were from Wako Pure Chemical Company, LTD, Japan.

Adsorption of ϕX174 by *E. coli* K12 and to its Ter-mutant strains :
The kinetics of attachment to whole bacteria were determined in a starvation buffer, as detailed by newbold and Sinsheimer (10).

Inactivation of ϕX174 phage by LPS from *E. coli* K12 and from its Ter-mutant strains : Bacteria were grown in the synthetic medium (9) with aeration at 37°C. 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 (11) and of Westphal et al (12).

The 50% inactivation doses (ID_{50} ;see 13) were estimated by titrating the residual infectivity after incubation (1 hr at 37°C) of increasing amounts of homogenized LPS with 5 X 10^4 PFU per ml starvation buffer, or of Simmon's medium containing magnesium ion.

Isolation and analysis of LPS core oligosaccharides : The LPS samples were degraded by heating (2 hr at 100°C) in 1% aqueous acetic acid, the insoluble lipid A was removed by centrifugation, and the core oligosaccharides were chromatographed through Biogel P2 (14).

The aldose analysis of the core oligosaccharides was carried out on the methyl-sugar derivatives formed after methanolysis with 1 N HCl-methanol for 6 - 8 hr at 100°C by gas-liquid chromatography (Shimadsu Gas Chromatograph, model GC 6 AM, column 2 m, 2.6 mmID, OV-17, 2%, chromosorb W, 80 - 100 mesh, carrier gas N_2, 60 ml/min, 2 Kg/cm^2, Temp. 150°C) and by thin-layer chromatography (Silica Gel 60 F_{254}, TLC plates, E. Merck AG) with solvent ; chloroform : methanol (3 : 1, v/v).

2-keto-3-deoxymannulosoctonic acid (KDO) was determined by the thiobarbituric acid method of Weissbach and Hurwitz (15), as modified by Osborn (16).

Heptose was determined by the cysteine-H_2SO_4 reaction as modified by Osborn (16).

The phosphorous determinations were carried out according to Lowry et al (17).

Results and Discussion

The susceptibility of *E. coli* K12 W2252-11U$^-$ and its Ter mutants to ϕx174 phage is shown in Table 1. *E. coli* K12 W2252-11U$^-$ (wild types) strains are non-sensitive to ϕx174 phage. The Ter-15 mutant derived from *E. coli* K12 (wild type I) becomes sensitive to the phage, but the Ter-21 mutant from *E. coli* K12 (wild type II) is insensitive. It is surmised from these results that the cell surface of the Ter-mutant could differ from that of the wild type cells. If the *E. coli* K12 (wild type) cells are a smooth or semirough chenotype, the Ter-mutant cells may mutate to a rough or "deep-rough" chenotype (18). Therefore, the Ter-mutant cells should show altered cell surface structures compared with those of the wild type cells under identical conditions of exponential cell growth.

Jazwinski et al (4) have reported that the core component of LPS of the outer cell membrane is the receptor for ϕx174 and its related phages. We have extracted the LPS from *E. coli* K12 (wild type) and its Ter-mutant cells, and measured the inactivity of ϕx174 phage by these LPS. The results show in Table 1 that the LPS from the Ter-15 mutant inactivates the phage, but the LPS from the Ter-21 mutant and parent cells (wild type I and II) cannot inactivate the phage. The LPS from *E. coli* C cells as a control inactivates the phage.

Further, we determined the nature of the sugars present in the core oligosaccharide of the LPS from *E. coli* K12 (wild types) and the Ter-mutant cells. Table 2 shows that sugar residues of the core oligosaccharide in the

Table 1. Susceptibility to ϕX174 phage and inactivation of ϕX174 by LPS.

Bacterial Strains	*Whole cells	Lipopoly-**saccharides
E. coli K12 (wild type I)	-	-
Ter-15	+	+
E. coli K12 (wild type II)	-	-
Ter-21	-	-
E. coli C	+	+

* ; (+) : sensitive, (-) : non-sensitive

** ; (+) : inactivated, (-) is not inactivated

LPS from the Ter-15 mutant occur in the same amounts as those in the LPS from *E. coli* K12 (wild type I), but the sugar residues of the core oligosaccharide in the LPS from the Ter-21 mutant are different from those in the LPS from *E. coli* K12 (wild type II).

The LPS from *E. coli* K12 are termed R antigens. They consist of lipid A and the core oligosaccharide, which may be complete in the *E. coli* K12 (R (rfb) mutants) cells (19,20). Because of the common occurrence of glucosamine, glucose, galactose, heptose and KDO from the core, these sugar components were termed basal sugars (21 - 24).

The results of analysis of core oligosaccharides in the LPS from *E. coli* K12 (wild types) and its Ter-mutant cells show that galactose, glucose,

Table 2. Molar ratios of the components of core fractions from the LPS of *E. coli* K12 and its Ter-mutants.

Bacterial Strains	Galactose	Glucose	Ribose	Heptose	KDO	Phosphorus
E. coli K12 (wild type I)	1.0	2.2	-	2.2	1.4	1.9
Ter-15	1.0	2.0	-	1.9	1.5	1.9
E. coli K12 (wild type II)	1.0	2.1	-	2.2	1.3	2.0
Ter-21	-	2.2	1.0	0.004	0.5	2.6
E. coli C	1.0	2.3	-	1.03	1.8	1.7

heptose and KDO are 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 are present. Neither galactose nor heptose can be found in the core from the Ter-21 mutant. The absence of these sugars explains why the Ter-21 mutant is non-sensitive to φx174 phage infection, because the galactose residue in the core is necessary for the phage adsorption (14). However, as the galactose content of the core of the Ter-15 mutant is the same amount as in the core from *E. coli* K12 (wild type) cells, the linkage between galactose and glucose in the core from the Ter-15 mutant may be different from that in the core from the wild type cells. The structure of the core oligosaccharide is now under investigation.

From the experiment with other core specific phage C21, the susceptibility of *E. coli* rough mutants to C21 has been shown to depend upon the absence of, or a reduction in, the relative amount of core galactose (25 - 27); in addition, the presence of heptose was found to be important (27). *E. coli* CR34 (28), which has little galactose in its LPS core and a substantial complement of heptose, is susceptible to C21, whereas *E. coli* GR 467, which has only 30% of the heptose present in strain CR 34, is not (29).

To study the alterations in the outer membrane of the cell envelope, heptose-deficient mutant cells were isolated from *E. coli* (28). The outer membrane of these "heptose-less" mutants contains drastically reduced amounts of the major outer membrane proteins, as the mutation in LPS biosynthesis apparently inhibits the incorporation of proteins into the outer membrane (28,18). From these reports, the Ter-21 mutant cells should have an altered outer membrane because of the reduction of the protein fraction, as the cells show an elliptical form after UV-irradiation, in contrast to the filament formation in other strains after UV-irradiation (30).

D-ribose residue is also found in the O-antigens O5 and O20 of *E. coli* strains (31,21), and the O5 antigen is made up of 3-amino-3,6-dideoxyglucose, galactosamine, galactose and ribose. The O20 antigen also is made up of

galactose and ribose, but the R-antigen of the Ter-21 mutant contains glucose, ribose and KDO. The content of ribose in the LPS of the Ter-21 mutant differs from that in O-antigens of *E. coli* and the mechanism of incorporation of D-ribose into the LPS of *E. coli* are now under investigation.

References

1. Sanger,F., Air,G.M., Barrell,B.G., Brown,N.L., Coulson,A.R., Fiddes,J.C., Hutchison III, C.A., Slocombe,P.M. and Smith,M. (1977) Nature 265, 687 - 695.
2. Sinsheimer,R.L. (1968) Progr. Nucl. Acid Res. 8, 115 - 169.
3. Lindberg,A.A. (1973) Annu. Rev. Microbiol. 27, 205 - 241.
4. Jazwinski,S.M., Lindberg,A.A. and Kornberg,A. (1975) Virology, 66, 268 - 282.
5. Fujimura,R. and Kaseberg,P. (1962) Biophys. J. 2, 433 - 449.
6. Brown,D.T., Mackenzie,J.M. and Bayer,M E. (1971) J. Virol. 7, 836 - 846.
7. Jazwinski,S.M. and Marco,R (1973) Fed. Proc. 32, 491.
8. Incardona,N.L. and Selvide,L. (1973) J. Virol. 11. 775 - 782.
9. Ohkawa,T. (1976) Europ. J. Biochem. 61, 81 - 91.
10. Newbold J.E. and Sinsheimer,S.L. (1970) J. Virol. 55, 427 - 431.
11. Galanos, C., Lüderitz,O. and Westphal,O. (1969) Europ. J. Biochem. 9, 245 - 249.
12. Westphal,O,, Lüderitz,O. and Bister,F. (1952) Z. Naturforsch. 76, 148 - 155.
13. Benmer,J. and Dirkx,J. (1960) Ann. Ins. Pasteur (Parie) 98, 910 - 914.
14. Feige,U. and Stirm,S. (1976) Biochem. Biophys. Res. Comm. 71, 566 - 573.
15. Weissbach,A. and Hurwitz,J. (1959) J. Biol. Chem. 234, 705 - 709.
16. Osborn,M.J. (1963) Proc. Nat. Acad. Sci. U.S.A. 50, 499 - 560.
17. Lowry,O.H., Roberts,N.R., Leiner,K.Y., Wu,M.L. and Farr,A.L. (1954) J. Biol. Chem. 207, 1 - 17.
18. Smit,J., Kamio,Y. and Nikaido,H. (1975) J. Bacteriol. 124, 942 - 958.
19. Schmidt,G. (1973) J. Gen. Microbiol. 77, 151 - 160.
20. Schmidt,G., Jann,B. and Jann,K. (1969) Europ. J. Biochem. 10, 501 - 510.
21. Orskov,I., Orskov,F., Jann,B. and Jann,K. (1977) Bacteriol. Rev. 41, 667 - 710.
22. Lüderitz,O., Jann,K. and Wheat,R. (1968) Compr. Biochem. 26A, 105 - 228.
23. Lüderitz,O. Westphal,O., Staub,A.M. and Nikaido,H. (1971) p145 - 233, In Weinbaum,G., Kadis,S. and Ajl,S.S. (ed), Microbiol Toxins, Vol. IV. Academic Press Inc., London.
24. Lüderitz,O., Galanos,C., Lehmann,V., Nurminen,M., Rietscgel,E., Rosenfelder,G., Simon,M. and Westphal,O. (1973) J. Infec. Dis. 128, (suppl), 9 - 21.
25. Kalcker,H.M., Laursen,P. and Rapin,A.M.C. (1966) Proc. Nat. Acad. Sci. U.S.A. 56, 1852 - 1858.
26. Rapin,A.M.C., Kalcker,H.M. and Alberico,L. (1968) Arch. Biochem. Biophys. 128, 95 - 105.
27. Schmidt,G., Jann,B. and Jann,K. (1970) Europ. J. Biochem. 16, 382 - 392.
28. Koplow,J. and Goldfine,H. (1974) J. Bacteriol. 117, 527 - 543.
29. Rooney,S.A., Goldfine,H. and Sweeley,C.C. (1972) Biochim. Biophys. Acta 270, 289 - 295.
30. Ohkawa,T. (1977) Biochim. Biophys. Acta 476, 190 - 202.
31. Vasilieu,N.N. and Zakharova,I.Y. (1976) Bioorg. Chem. 2, 199 - 206.