Bacteriophage-resistant Mutants of *Escherichia coli* K12. Location of Receptors within the Lipopolysaccharide

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A series of mutants of *Escherichia coli* K12 resistant to lipopolysaccharide (LPS)-specific bacteriophages were isolated, and examined with regard to their general properties, phage typing, chemical analysis of their LPS, and genetic analysis. Fourteen classes of mutants were distinguished on the basis of phage typing and sensitivity to bile salts. Three of the mutant classes are sensitive to phages to which the parent is resistant. Mutants which are sensitive to bile salts generally lack heptose in their LPS, but two mutant classes are exceptions to this rule. Analyses of the sugars in the purified LPS of all mutant classes indicated that mutants were obtained which are blocked at most stages in core polysaccharide synthesis. On the basis of the chemical analysis, in conjunction with phage typing data and other known properties of the mutants, it is deduced which residue(s) is involved as a receptor for each of the phages used and which residues hinder these receptors. Some of the mutant classes do not seem to be changed in their LPS structure. Many of the mutations map in or near the *rfa* locus, but some are far removed from this region.

INTRODUCTION

It has long been known that the lipopolysaccharide (LPS) of certain Gram-negative bacteria serves as a receptor for the adsorption of many so-called LPS-specific bacteriophages. In recent years specific residues in the polysaccharide moiety of the LPS have been identified as the receptor (Lindberg, 1973; Prehm *et al.*, 1976*a*). It is not known, however, whether other specific components of the cell surface may be involved in injection and penetration of DNA. For example, Bayer (Bayer, 1968*a, b*; Bayer & Starkey, 1972; Bayer, 1975) has identified sites of adhesion between the inner and outer membranes of *Escherichia coli* at which many phages (including LPS-specific phages such as T4 and ϕ X174) are observed to adsorb exclusively. It has also been suggested that the cytoplasmic membrane may be involved in infection of *E. coli* B by phage T4 (Simon & Anderson, 1967; Wais & Goldberg, 1969; Dawes, 1976), although glucose residues in the LPS are necessary for adsorption (Prehm *et al.*, 1976*a*).

One approach to this problem is to identify components of the cell surface involved in adsorption and penetration of DNA, by isolating mutants which lack them. It is necessary to know which residues in the LPS are important as receptors for a given phage, and what array of mutant genotypes are obtained as a result of loss of these sugar residues. Although such information is available for some bacteria, particularly *Salmonella typhimurium* and *E. coli* B (Lindberg, 1973; Prehm *et al.*, 1976*a*), we have used *E. coli* K12 to facilitate genetic

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Table 1. *Characteristics of strains*

Strain	Genotype* or phenotype	Source/reference
<i>Escherichia coli</i> K12		
AB1157	F ⁻ <i>thr-1 ara-14 leu-6 proA2 lacY1 tsx-35 supE44 galK2 his-4 rpsL xyl-5 mtl-1 argE3 thi-1 λ⁻ sup-37</i>	Bachmann (1972)
AB1157-W30	F ⁻ <i>thr-1 ara-14 leu-6 supE44⁺? his-4 xyl-5 rpsL mtl-1 argE3 thi-1 λ⁻ sup-37</i>	From AB1157 by conjugation with HfrB7 and selection for Pro ⁺
AB1157-W31	F ⁻ <i>thr-1? ara-14? leu-6? supE44⁺? galEΔ209 rpsL xyl-5 mtl-1 argE3 thi-1 λ⁻ sup-37</i>	From AB1157 by conjugation with HfrH209 and selection for His ⁺
K1060-18T1	F ⁻ <i>thi fadE rpsL galEΔ209</i>	Beacham & Silbert (1973)
H209	HfrH <i>thi galEΔ209</i>	S. Adhya
<i>Salmonella typhimurium</i>		
TV119	<i>rfb-430</i>	B. A. D. Stocker (Wilkinson & Stocker, 1968)

* Symbols are as recommended by Bachmann *et al.* (1976).

studies and also because it was of interest to identify receptors in this organism. In this study we report a collection of phage-resistant mutants whose analysis aids in the identification of specific sugar residues as receptors for adsorption and of other residues which hinder these receptors. Most of the mutants are variously deficient in their LPS,¹ but some may not be.

METHODS

Bacterial strains are listed in Table 1. The origins of transfer of Hfr strains used are given in Fig. 1 (see Low, 1972; Bachmann, Low & Taylor, 1976).

Bacteriophages are listed in Table 2.

Media and growth of cultures. Mineral medium was M9 (Miller, 1972) containing 0.2% (w/v) glucose and appropriately supplemented with amino acids (20 µg ml⁻¹) and vitamins (10 µg ml⁻¹). Medium containing bile salts was either MacConkey agar (Oxoid) or R broth containing sodium tauroglycocholate (5 g l⁻¹; Oxoid). R broth contained (per litre): 10 g tryptone, 5 g NaCl, 1 g glucose and 1 g yeast extract. Cultures were grown at 37 °C, and were usually aerated by shaking in a New Brunswick gyrotary shaker. However, for extraction of lipopolysaccharide (LPS), bacteria were grown in 10 l M9 medium containing 0.2% (w/v) Casamino acids (Difco), and were aerated by passing filtered air through sintered glass blocks immersed in the cultures.

Isolation of lipopolysaccharide. LPS was extracted with phenol/chloroform/petroleum ether (PCP) and purified, according to Galanos, Lüderitz & Westphal (1969).

Mild acid hydrolysis of lipopolysaccharide. LPS (15 mg) was subjected to mild acid hydrolysis as described by Schmidt, Jann & Jann (1969), using 1% (v/v) acetic acid.

Sugar determinations. Polysaccharide solutions prepared from PCP-extracted LPS by mild acid hydrolysis were reduced to monosaccharides by hydrolysis for 1 h at 121 °C with 2 M-trifluoroacetic acid (TFA) as described by Albersheim *et al.* (1967). After cooling, the TFA was removed in a stream of filtered air at 37 °C. The residue was taken up in 0.5 ml distilled water, and brought to neutrality by the addition of 0.05 ml 0.25 M-sodium phosphate buffer, pH 7.0.

Glucose was determined using glucose oxidase (Sigma glucostat). *N*-Acetylglucosamine was measured as described by Reissig, Strominger & Leloir (1956). L-Glycero-D-manno-heptose was determined according to Osborn (1967) and rhamnose was measured in the same assay by the method of Dische (1955). D-Glycero-L-manno-heptose, used as a standard, was obtained from E. Zissis (National Institutes of Health, U.S.A.). 2-Keto-3-deoxyoctonic acid (KDO) was determined with the thiobarbituric acid reagent according to Warren (1959), using sialic acid as a standard. Phosphate was measured as described by Ames & Dubin (1960).

Polysaccharide obtained by mild acid hydrolysis of LPS was hydrolysed with TFA (containing inositol as an internal standard), as described above. The residues obtained were dried and converted to alditol acetates as described by Albersheim *et al.* (1976). The alditol acetates were then separated by gas-liquid chromatography.

Conjugation. Bacteria were grown to late-exponential phase (2×10^8 organisms ml^{-1}) in R broth. Male strains were grown without shaking. The donor (1 ml) and recipient strains (10 ml) were mixed and allowed to stand at 37 °C for 1 to 2 h. Samples, appropriately diluted, were then spread on selective media containing streptomycin to counterselect the male. After purification, 60 to 100 recombinants were scored for unselected markers. For testing phage sensitivity, this was performed by cross-streaking the appropriate phage with each recombinant on R broth agar; for testing bile-salt resistance/sensitivity, recombinants were streaked on MacConkey agar.

Isolation and properties of mutants

Since strains lacking heptose in their LPS are sensitive to bile salts (Wilkinson, Gemski & Stocker, 1972; see also Mäkelä & Stocker, 1969; Stocker & Mäkelä, 1971), presumably due to increased permeability of the outer membrane, all mutants were tested for this character (Table 4). Strains C35, F6s and T4338, which lack heptose (see below), are unable to form colonies on medium containing bile salts. However, strain BN7 is sensitive to bile salts but does not lack heptose, and strain BN30 is deficient in heptose but is not sensitive to bile salts.

The strains listed in Table 3 were also examined for their ability to be agglutinated by plant lectins; the results are reported in detail by Picken & Beacham (1977). It may be noted here, however, that Concanavalin A did not agglutinate the parent strain AB1157-W30, but did agglutinate mutant classes 2, 3 and 5. The class 2 strain was only weakly agglutinated,

Table 2. *Origin and characterization of bacteriophages*

Phage	Host	Surrogate host	Origin	Source
U ₃	<i>E. coli</i> K12 W3110	ABI157-W30	Watson & Paigen (1971)	K. Paigen
6SR	<i>S. paratyphi</i> A	<i>S. typhimurium</i> TV119	Felix, 1948, see Kay (1962)	B. A. D. Stocker
Br ₂	<i>S. paratyphi</i> B Kröger I/2	U440	Brandis (1966)	B. A. D. Stocker
C ₂₁	<i>E. coli</i> B	U110	Burnet & McKie (1933)	M. J. Osborn
Br ₁₀	<i>S. paratyphi</i> B Kröger I/2	ABI157-W30	Brandis (1966)	G. Schmidt
Br ₁₀	<i>S. paratyphi</i> B Kröger I/2	ABI157-W30	Brandis (1966)	H. Brandis
FP ₁	<i>S. minnesota</i> R5	ABI157-W30	Isolated from R5, Schmidt & Lüderitz (1969)	G. Schmidt
T ₁	<i>E. coli</i> B or <i>E. coli</i> K12	ABI157-W30		S. E. Luria
T ₃		ABI157-W30		M. Matsushashi
T ₄		ABI157-W30		R. Buxton, E. Yagil
T ₅		ABI157-W30		R. Buxton
T ₆		ABI157-W30		B. Wilkins
T ₆		ABI157-W30		M. Matsushashi
T ₇		ABI157-W30		

Table 3. *Phage type and bile-salt sensitivity of all mutant classes obtained from ABI157-W30*

Phage sensitivity (+ = sensitive; - = resistant) was tested by cross-streaking the phage with each mutant strain. (+) indicates partial sensitivity in such a test, which, unless indicated otherwise, is equivalent to an efficiency of plating (e.o.p.) of $< 10^{-3}$. Bile-salt sensitivity (BS) is indicated by: +, sensitive; -, resistant.

Class	Strain	U ₃	6SR	Br ₂	C ₂₁	Br ₁₀	FP ₁	BS	T ₃	T ₄	T ₇	T ₁	T ₅	T ₆
1	ABI157-W30	+	-	-	-	+	+	-	+	+	+	+	+	+
2	ABI157-W31	-	-	(+)	+	+	+	-	+	+	+	+	+	+
3	U440	-	+	+	(+)	+	+	-	+	+	+	+	+	+
4	U ₂	-	-	-	+	+	+	-	+	+	+	+	+	+
5	U1020	-	-	+	+	-	+	-	+	+	+	+	+	+
6	U110	-	-	-	+	(+)	+	-	(+)	+	+	+	+	+
7	C35*	-	-	-	-	-	-	+	(+) [†]	+	+	+	+	(+)
8	F68	-	-	-	-	-	-	-	(+)	+	-	+	+	+
9	T4203	+	-	-	-	+	+	-	+	-	+	+	+	+
10	T4235	-	-	-	-	+	+	-	+	-	+	+	+	+
11	T4204	-	-	-	+	+	+	-	(+)	-	+	+	+	(+)
12	T4338	-	-	-	-	-	-	+	+	-	+	+	+	-
13	BN7	+	-	-	-	-	+	+	+	+	+	+	+	+
14	BN30	-	-	-	-	-	-	-	(-)	-	+	+	+	-
15	BSP23	+	-	-	-	-	+	-	+	+	+	+	+	+

* The parent of this mutant is a U₃-resistant C₂₁-sensitive mutant of ABI157-W30.

[†] e.o.p., 0.1. [‡] e.o.p., 0.18. § e.o.p., 1.6×10^{-4} , although positive in a cross streak.

Table 4. Viability of mutant strains of AB1157-W30 on MacConkey agar

Organisms of each strain were plated in soft agar on R broth and MacConkey agar, and the viability on MacConkey agar is given as a percentage of that on R broth agar.

Strain	% Viability	Strain	% Viability
AB1157-W30	100	T4203	104
U440	105	T4235	70
U2	177	T4204	60
U1020	60	T4338	< 10 ⁻³
U110	59	BN7	< 10 ⁻³
C35	< 10 ⁻³	BN30	124
F6S	< 10 ⁻³	BSP23	66

Fig. 2. Proposed structure of the LPS of *E. coli* K12 (strain AB1157-W30) and of various mutants. Dashed lines indicate a tentative or unknown linkage. The galactose residue in parentheses indicates the postulated presence of very small amounts of this residue, which is not evident from quantitative analysis. Strain U440 is shown with two glucose residues on the basis of its phage type and agglutination by Concanavalin A, though the analysis of LPS indicates an average glucose content of between two and three residues. Gal, galactose; Glc, glucose; Hep, heptose; Rha, rhamnose; KDO, 2-keto-3-deoxyoctonate.

and another *galE* strain (K1060-18T1) containing the same mutant allele did not agglutinate. Since Concanavalin A interacts with terminal glucose residues (or mannose or *N*-acetylglucosamine) (Goldstein & Iyer, 1966), and glucose is present in the core region of the LPS of *E. coli* K12 (Figs 2, 3), we conclude that terminal glucose residues are exposed in these mutant strains, but not in the parent strain. *Salmonella typhimurium* strain TV119, which has a terminal *N*-acetylglucosamine residue, was also agglutinated by Concanavalin A.

Strains T4203 and U440 have similar molar ratios of sugars in their LPS (see later), and yet have greatly differing properties (Table 3; Picken & Beacham, 1977). If T4203 contains a less abbreviated LPS structure than U440, it may be predicted that U₃-resistant mutants with the phage type and other properties of strain U440 should be obtainable from strain T4203, but that T₄-resistant mutants with the phage type of strain T4203 should not be readily derived from U440 since this would require two mutational steps. This prediction has been confirmed.

All the mutant strains in Table 3 were isolated with reasonably high frequency (> 5%), except class 15 in which only a single mutant (strain BSP23) was found.

Fig. 3. Structure of LPS of *E. coli* K12 as proposed by (a) Benedetto *et al.* (1976) and (b) Prehm *et al.* (1976a). Dashed lines indicate uncertain linkage. It is proposed that GlcNAc (in b) is not present on all core chains. GlcNAc, *N*-acetylglucosamine; Etn, ethanolamine; other abbreviations as in Fig. 2.

Table 5. *Composition of polysaccharide isolated from LPS obtained from parent and mutant strains based on enzymic and chemical analysis*

Results are expressed as molar ratios relative to KDO, assigned as discussed in the text. Approximate molar ratios are given in parentheses. GlcNAc, *N*-acetylglucosamine; Rha, rhamnose; Glc, glucose; Hep, heptose; KDO, 2-keto-3-deoxyoctonic acid.

Strain	Molar ratio relative to KDO					
	GlcNAc	Rha	Glc	Hep	KDO	PO ₄ ³⁻
ABI157-W30	0.04(0)	0.29(0.3)	3.03(3)	6.94(6)*	1.45(1 or 2)	4.96(5)
U440	0.07(0)	0.26(0.3)	2.43(2)	6.10(6)	1.45(1 or 2)	4.44(4)
U2	0.07(0)	0.19(0.2)	1.13(1)	5.09(5)	1.45(1 or 2)	2.38(2)
UI020	0.02(0)	0.16(0.2)	1.09(1)	2.61(3)	1.45(1 or 2)	1.61(2)
UI10	0.01(0)	0.11(0.1)	0.03(0)	3.03(3)	1.45(1 or 2)	1.39(1)
C35	0.05(0)	0.16(0.2)	0.08(0)	0.08(0)	1.45(1 or 2)	1.28(1)
F68	0.08(0)	0.15(0.2)	0.09(0)	0.06(0)	1.45(1 or 2)	1.15(1)
T4203	0.05(0)	0.24(0.2)	1.91(2)	6.28(6)	1.45(1 or 2)	3.71(4)
T4235	0.04(0)	0.19(0.2)	1.06(1)	5.59(6)	1.45(1 or 2)	2.07(2)
T4204	0.02(0)	0.13(0.1)	0.07(0)	3.28(3)	1.45(1 or 2)	1.73(2)
T4338	0.04(0)	0.16(0.2)	0.03(0)	0.08(0)	1.45(1 or 2)	1.27(1)
BN7	0.02(0)	0.22(0.2)	2.45(2 or 3)	6.41(6)	1.45(1 or 2)	4.10(4)
BN30	0.08(0)	0.16(0.2)	0.30(0)	0.74(1)	1.45(1 or 2)	1.75(2)
BSP23	0.04(0)	0.20(0.2)	2.04(2)	5.55(6)	1.45(1 or 2)	3.68(4)

* In view of previous data (see text) indicating a maximum of six, not seven, heptose residues, we interpret this value as also indicative of the presence of six heptose residues in the parent strain.

Chemical analysis

We expected that most phage-resistant mutants would be variously deficient in their LPS, according to the residues involved as the receptor for any particular phage. Conversely, an analysis of the LPS of any mutants altered primarily in some other component of the cell surface should provide an indication that it is the same as the parent strain. The molar ratios of LPS sugars were therefore determined in polysaccharide derived from purified LPS by mild acid hydrolysis (see Methods). The results of enzymic and chemical analyses are shown in Table 5 (galactose was not included in these assays). The ratio of glucose:heptose in the parent strain is given as 3:6 since this has previously been determined by similar methods for *E. coli* K12 strains (Rooney & Goldfine, 1972; Boman & Monner, 1975; Benedetto, Bruneteau & Michel, 1976). The mutants appear to lose glucose in units of one, whereas they seem to have either six, five, three, or no heptose residues. Phosphate also

Table 6. *Composition of polysaccharide isolated from LPS obtained from parent and mutant strains, based on gas-liquid chromatography*

Results are expressed as molar ratios relative to galactose, except for U1020 which is considered to possess a single glucose residue. The heptose values are corrected for destruction as described in Methods. Since destruction of heptose is probably variable these values are considered less reliable, as an indication of mutational loss of heptose, than those in Table 5 and are therefore only approximate. Approximate molar ratios are given in parentheses. For strains containing heptose only (U110 and T4204), the analysis results relative to the internal standard are consistent with a molar content of about 3. Abbreviations are as in Table 5, except Gal, galactose.

Strain	Molar ratio relative to galactose		
	Gal	Glc	Hep (corrected)
AB1157-W30	1.0(1)	3.26(3)	4.31(4)
U440	1.0(1)	2.57(2 or 3)	3.29(3)
U2	1.0(1)	1.06(1)	3.39(3)
U1020	0.06(trace)	1.0(1)	3.4(3)
U110	0	0	(3)
C35	0	0	0
T4203	1.0(1)	2.73(3)	2.59(3)
T4235	1.0(1)	1.14(1)	1.91(2)
T4204	0	0	(3)
T4338	0	0	0
BN7	1.0(1)	2.94(3)	2.12(2)
BSP23	1.0(1)	3.12(3)	2.51(3)

seems to be lost in a gradual manner. No *N*-acetylglucosamine was detectable, and rhamnose was present in low variable amounts.

The sugar analyses obtained by g.l.c. (Table 6) indicate that galactose is present to the extent of one-third the amount of glucose. In certain mutants galactose is absent. In strains U440, U2 and T4235 glucose has been lost without detectable loss of galactose; in strain T4235 there are only single glucose and galactose residues. Assuming that glucose residues are not present as side-chain residues (Benedetto *et al.*, 1976; Prehm *et al.*, 1976*b*), these results suggest that the galactose residue is not terminal. The g.l.c. analysis, however, differs from that shown in Table 5 in that only three heptose residues relative to galactose and glucose were detected in the parent strain. Benedetto *et al.* (1976) also found six residues by chemical assay and three by g.l.c. analysis (see below).

Genetic characterization

Many of the strains in Table 3 have been mapped in a preliminary manner by linkage analysis. The main purposes of this were twofold. Firstly, as will be discussed below, the four classes of T4-resistant mutants (classes 9 to 12) have phage types identical or very similar (apart from T4 resistance) to the parent strain (class 1) and classes 4, 6 and 8 respectively, which were selected for resistance to other phages. We wished, therefore, to see if we could demonstrate that two apparently similar mutants mapped in different loci. Secondly, we wanted to determine whether any of the genes identified in this study mapped near the *rfa* locus (Stocker & Mäkelä, 1971; Schmidt, 1973; Bachmann *et al.*, 1976; Hancock & Reeves, 1976).

Crosses using HfrP4X (Table 7) showed that the mutations in strains U2, U110 and T4235 are linked to the *xyl* and *mtl* loci (83 to 95%) and are thus probably in the *rfa* locus. The mutation in T4338 also shows closer linkage to *xyl* (60%) than to other markers (Table 8) but to a lesser degree than the mutations in strains U2, U110 and T4235. All of 70 bile-salt resistant recombinants tested were sensitive to T4, indicating that the T4 resistance and bile-salt sensitivity of strain T4338 are due to the same genetic lesion. No linkage to the

Table 7. *Analysis of recombinants from crosses between HfrP4X and various mutant strains*

Results show the percentage of colonies of each selected phenotype that also had the unselected phenotype. The unselected phenotypes were: U₃^s, U₃ sensitivity; T₄^s, T₄ sensitivity; BS^R, bile-salt resistance. Other genetic symbols are as in Bachmann *et al.* (1976).

Recipient	Unselected phenotype	Selected phenotype							
		Leu ⁺	Thr ⁺	Arg ⁺	Mtl ⁺	Xyl ⁺	His ⁺	Gal ⁺	Nad ⁺
U2	U ₃ ^s	2	2	3	90	87	16		
U110	U ₃ ^s	0	0	2	83	30	13		
F6s	BS ^R	76							
T4203	T ₄ ^s	0	0	0	3	7	56		
T4235	T ₄ ^s	2	2	10	95	93			
T4204	T ₄ ^s	0	0	0	0	2	63	100	14
T4338	T ₄ ^s and BS ^R	0							

Table 8. *Analysis of bile-salt resistant recombinants from crosses with HfrP4X*

Results show the percentage of colonies having the unselected phenotype. Symbols as in Table 7.

Unselected phenotype	Recipient strain	
	F6s	T4338
Leu ⁺	42	39
Thr ⁺	35	44
Arg ⁺	7	37
Xyl ⁺	2	60
His ⁺	0	0
T ₄ ^s		100

Table 9. *Analysis of recombinants from crosses between HfrH and mutant strains*

Results show the percentage of colonies of each selected phenotype that also had the unselected phenotype. Symbols as in Table 7.

Recipient	Unselected phenotype	Selected phenotype					
		Thr ⁺	Leu ⁺	His ⁺	Xyl ⁺	Gal ⁺	Nad ⁺
F6s	BS ^R		78				
T4203	T ₄ ^s	0	0	26	5		
T4204	T ₄	0	1	51	38	100	47

leu gene was observed when either Leu⁺ or bile-salt resistant recombinants were selected; this is in contrast to the mutation in another 'heptose-less' strain, F6s.

In crosses between strain F6s and HfrP4X or HfrH, 76% and 78% of Leu⁺ recombinants, respectively, were bile-salt resistant (Tables 7 and 9). Consistent with this, 35% and 42% of bile-salt resistant recombinants became Leu⁺ or Thr⁺ in the same cross with HfrP4X, but only 2% became Xyl⁺ (Table 8). Hence the mutation in f6s leading to bile-salt sensitivity is near the *thr* and *leu* loci, and is unlinked to *xyl*.

When T4203 was crossed with HfrP4X, 56% of His⁺ recombinants were T₄-sensitive (Table 7). Less than 10% of all other recombinants were T₄-sensitive. Hence, the site of the T4203 mutation must be near *his*. The results of a cross with HfrH (Table 9) show that 26% of the His⁺ recombinants, compared with 5% of Xyl⁺ recombinants, became T₄-sensitive, tending to confirm that the gene is near the *his* locus and not near *xyl*. This may also indicate that the mutation is to the left of *his* since closer linkage is observed with HfrP4X. In a similar cross between strain T4203 and HfrKL99, 35% of the His⁺ recombinants, but only 15% of Xyl⁺ recombinants, were T₄-sensitive (Table 10), in agreement with the results from the cross with HfrH.

Table 10. *Analysis of recombinants from crosses between HfrKL99 and T4203 or T4204*

Results show the percentage of colonies of each selected phenotype having the unselected phenotype, T4 sensitivity. Symbols as in Table 7.

Recipient	Selected phenotype			
	Gal ⁺	His ⁺	Xyl ⁺	Nad ⁺
T4203		35	15	
T4204	100	41	0	35

Strain T4204 is unable to ferment galactose (Gal⁻) and has a specific requirement for nicotinic acid (Nad⁻). We have found that *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine will induce Nad⁺ revertants but not Gal⁺ revertants, and therefore these mutations do not appear to result from a deletion of both *nad* and *gal* genes. Of 60 Nad⁺ revertants tested, all retained the phage type of T4204 and were Gal⁻. While the *nad* mutation seems to be a point mutation, the *gal* mutation, being non-revertible, may be a deletion. Crosses reported below confirm that the strain is a double mutant, though this is very unexpected since T4204 arose spontaneously.

In a cross between strain T4204 and HfrP4X (Table 7), all the Gal⁺ recombinants were T4-sensitive, suggesting that the two mutations are extremely close or identical. That 63% of His⁺ recombinants were T4-sensitive suggests that the site of the mutation is near *his*. In a similar cross using HfrH (Table 9), again all Gal⁺ recombinants (and 51% of His⁺ recombinants) were T4-sensitive. These data support the results of the previous cross. Since all the Gal⁺ recombinants from both crosses were T4-sensitive, the mutation resulting in T4 resistance is probably in either the *galU* or *galE* gene. To distinguish between these loci, T4204 was crossed with HfrKL99, whose point of origin is between the *galE* and *galU* genes (Fig. 1). If the mutation is in *galU*, a large number of Gal⁺ recombinants, relative to other genes, should be obtained since *galU* is proximal to the origin, but few, if any, should be obtained if the mutation is in the distal *galE* gene. About three times as many Gal⁺ recombinants as His⁺ and Xyl⁺ recombinants were obtained, indicating that the mutation is in the *galU* gene: again 100% of the Gal⁺ recombinants were T4-sensitive (Table 10). (The fact that T4204 lacks glucose is consistent with the lesion being in the *galU* gene, since such mutants cannot synthesize UDPglucose).

DISCUSSION

A striking feature of the mutants is that some of them, in becoming resistant to one phage, acquire sensitivity to another. Thus the parent strain is resistant to phages 6SR, Br2 and C21, but class 3 is 6SR-sensitive, classes 3 and 5 are Br2-sensitive and classes 2, 5, 6 and 11 are C21-sensitive. The latter has been attributed to masking specifically by galactose of the C21 receptor (possibly heptose) which is unmasked in sensitive mutants lacking galactose (Rapin & Kalckar, 1971). It seems clear (see below) that the sensitivity to 6SR and Br2 arises from the exposure of a particular terminal glucose residue which is required as a receptor.

Four classes of mutant (9 to 12) were selected for resistance to phage T4 and all of these were defective in adsorption of the phage (data not shown). One class (9) was indistinguishable, on the basis of phage type, from the parent strain AB1157-W30, indicating that its LPS is unaltered. The chemical analysis and genetic evidence is consistent with this, though the enzymic assay of glucose content gives a low value. As noted above, classes 10 to 12 have counterparts (classes 4, 6 and 8 respectively) with regard to their phage type, expect that they are T4-resistant. The simplest explanation for this may be that they are more stringent

mutations in the same way that a T4-resistant *galU* *E. coli* B/4 mutant of Hattman & Fukasawa (1963) is a more stringent mutant than a T4-sensitive *galU* *E. coli* K12 mutant.

Previous work with phage Br10 has indicated that LPS is required as a receptor, but work with *S. minnesota* and *E. coli* O8 is contradictory (Schmidt & Lüderitz, 1969; Schmidt, Jann & Jann, 1970). Mutants resistant to phage Br10 (classes 5, 6 and 11) confirm that LPS is involved in the reception of this phage since they were isolated as resistant to an LPS-specific phage, U3 or T4, and they have an altered LPS. Classes 5, 6 and 7 have also been obtained by selection for Br10 resistance (unpublished results). The mutants BN7 and BSP23, however, are not obviously due to a mutation affecting the LPS. Both strains have the same phage type as the parent, indicating an unaltered LPS. The LPS analysis is generally consistent with this, though the enzymic assay of glucose is low for BSP23.

All mutants showing resistance to the majority of phages (C35, F6s, T4338) are unable to grow on medium containing bile salts. Additionally these strains are resistant to T3 and T7 (and some to T4), two phages known to have their receptors in the LPS (see Lindberg, 1973). These observations are consistent with the absence of all sugars distal to KDO (Tables 5 and 6; Wilkinson *et al.*, 1972; Lindberg, 1973). Strains lacking two or three glucose residues, and not completely deficient in heptose, are slightly sensitive to bile salts (Table 4). All strains, including those sensitive to bile salts, remain sensitive to the protein-requiring phages T1 and T5. However, strains T4338 and BN30 are resistant to the protein-requiring phage T6 (Michael, 1968; Verkleij, Lugtenberg & Ververgaert, 1976; Manning & Reeves, 1976). Ames, Spudich & Nikaido (1974) and Koplów & Goldfine (1974) have demonstrated that loss of heptose in the LPS can affect the protein composition of the outer membrane. Such an effect may be implicated here.

It has become axiomatic, in considering LPS mutants, that a highly abbreviated LPS, associated with the heptose-less condition, leads to bile-salt sensitivity (Wilkinson *et al.*, 1972; see also Mäkelä & Stocker, 1969; Stocker & Mäkelä, 1971). However, two classes of Br10-resistant mutants were isolated showing an unusual reaction to bile salts. Strain BN7, whilst retaining the overall phage type of AB1157-W30, is resistant to phage Br10 and sensitive to bile salts, and appears to have a complete LPS. In contrast, strain BN30 has the phage type of a heptose-less strain, is deficient in LPS heptose, and yet is resistant to bile salts.

As expected, many of the mutations are probably in the *rfa* locus since they are closely linked (83 to 95%) to the *xyl* and *mtl* loci (Schmidt, 1973; Bachmann *et al.*, 1976; Hancock & Reeves, 1976). The heptose-less mutant T4338 is less closely linked (60%) to *xyl*, consistent with it being the equivalent of the *rfaE* gene in *Salmonella typhimurium* (Stocker & Mäkelä, 1971). Less expected was the mapping of the mutations in strains F6s and T4203 in the *thr-leu* and *his* regions, respectively. Other mutations associated with cell envelope defects or phage resistance, defining the *lpcA* (Tamaki, Sato & Matsushashi, 1971), *envA* (Normark, Boman & Matsson, 1969; Normark, 1970) and *tfrA* (Curtiss, 1965) loci, have been mapped in the *thr-leu* region, but the location near *his* of a mutation (in strain T4203) specifying T4 resistance seems unique. Though near the *rfb* locus, *E. coli* K12 is already *rfb* (Ørskov & Ørskov, 1962; Jones, Koeltzow & Stocker, 1972), so that this would not seem to be the explanation. The unusual Br10-resistant strains (classes 13 to 15) have not yet been examined genetically.

Detailed structural analyses of the LPS of *E. coli* K12 have recently been carried out by Benedetto *et al.* (1976) and by Prehm *et al.* (1976b); the structures they propose are shown in Fig. 3. Although our quantitative analysis gives no direct structural information it raises two points. Firstly, we found six heptose residues relative to other sugars in agreement with others using similar methods (Rooney & Goldfine, 1972; Boman & Monner, 1975; Benedetto *et al.*, 1976). However, Benedetto *et al.* (1976) and Prehm *et al.* (1976b), using g.l.c. analysis, found only three or four heptose residues, respectively, relative to the galactose and glucose content. We have also found three heptose residues (Table 7), using a slightly different

Fig. 4. Proposed structure of LPS of *E. coli* K12 showing which residues serve as receptors for various phages. Abbreviations as in Fig. 2.

g.l.c. procedure. Although the reason for this is not clear, we suggest that the isolation of mutants with three rather than six heptose residues as measured by chemical analysis, coupled with the fact that such mutants retain three heptose residues as measured by g.l.c. analysis, lends credence to the hypothesis that the inner core contains more than three, and possibly six, residues. Since half of the heptose units seem so readily lost as a result of mutation, moreover with retention of hexose (strain U1020), these units may be present as a side-chain. Such an arrangement (Fig. 2) is similar to that proposed by Prehm *et al.* (1976b).

Secondly, the parent strain, AB1157-W30, is the only strain (other than the unusual mutants, T4203, BN7 and BSP23, which are presumed to be like the parent in their LPS composition) of those possessing LPS galactose that is sensitive to phage U3; other mutants are U3-resistant and retain the single (side-chain) galactose residue. Phage typing studies indicate that U3 requires a sugar occupying a terminal position in the LPS chain of *E. coli* K12 (see below). There is strong evidence in *E. coli* for galactose being the specific requirement for the adsorption of this phage (Watson & Paigen, 1971). These considerations, and the results of Benedetto *et al.* (1976), point to the presence of a terminal galactose residue in *E. coli*. We therefore postulate that the LPS of *E. coli* K12 contains two galactose residues: one attached to glucose I (Prehm *et al.*, 1976b; and see Results) and the other attached to glucose III. This latter residue, we suggest, is present in only trace amounts in AB1157-W30 (Fig. 2) since only one molecule was detected by analysis.

On the basis of Fig. 2, we can tentatively assign a structure to the LPS of most mutant classes, as deduced from the quantitative analyses and phage typing. Should the structures in Fig. 2 be confirmed by structural analysis, then LPS derived from them should prove useful substrates for enzymic studies on the biosynthesis of the LPS.

From the phage type of the mutant strains, the sugar analyses, and the structural work of Benedetto *et al.* (1976) and Prehm *et al.* (1976b), the sugar residues important in determining the phage receptors can be deduced. Where a change in the LPS is observed it is assumed to result in inefficient adsorption. However, lack of adsorption has not been demonstrated in all cases, and the more unusual mutants are excluded from consideration. A great deal of information is available on receptor requirements from work on other species of Enterobacteriaceae. In accord with such work on T4 (Jesaitis & Goebel, 1955; Beumer *et al.*, 1966; Dawes, 1975; Prehm *et al.*, 1976a), Br2 (Wilkinson & Stocker, 1968; Lindberg, 1973) and 6SR (see Lindberg, 1973), our data indicate that LPS glucose is involved in forming a receptor for these phages in *E. coli* K12. Thus sensitive strains have, on the basis of the known structure of LPS (Fig. 3, and see Fig. 2) and of their agglutination by Concanavalin A (Picken & Beacham, 1977), a terminal glucose residue. Strain U110 is an exceptional T4-sensitive strain, but may contain a 'leaky' mutation (see above).

However, it seems that the glucose I residue (and possibly more distal ones to a lesser extent) is 'masked' by side-chain heptose residues since strain U1020 is sensitive to Br2 and T4, whereas T4235 is resistant to both these phages, and U2 is Br2-resistant. Mutant U2 is T4-sensitive but probably lacks one heptose residue which may partially 'unmask' the glucose I residue.

Phage U3 was isolated on the basis of its specificity for the presence of galactose in the LPS of *E. coli* K12 (Watson & Paigen, 1971). As discussed above (Table 3; Fig. 2), only the parent strain, AB1157-W30 (apart from strains T4203, BN7 and BSP23) is U3-sensitive and results for several U3-resistant mutant classes (3 and 10) are consistent with their possessing an abbreviated LPS, but retaining the side-chain galactose residue. Also, Jones *et al.* (1972) have shown that the transfer of O antigen polysaccharide chains to *E. coli* K12 results in the masking of the U3 receptor. These findings are consistent with the requirement, by U3, of a terminal sugar residue in the core polysaccharide, for its receptor. That this terminal residue may be galactose is in agreement with the results of Benedetto *et al.* (1976).

The receptor for phage Br10 appears to be either the postulated side-chain heptose residues, since strain U1020 which lacks them is resistant, or heptose II, provided that it is unsubstituted by glucose. However, heptose II would seem to be a poor substitute for the side-chain heptoses since strains U110 and T4204 are only partially sensitive. This is not the case in *S. minnesota* where only Ra and Rb strains are sensitive to phage Br10 (Schmidt & Lüderitz, 1969), implying that Br10 requires an LPS glucose II residue in *S. minnesota*. Additionally, Schmidt *et al.* (1970) have reported that Br10-resistant strains of *E. coli* O8 lack LPS phosphate. This phage therefore appears to have different receptor requirements in different strains of enteric bacteria, though all reside in the LPS. The lesions in strains BN7 and BSP23 and hence their role in Br10 reception remain unknown.

Phage FP1 appears to have a non-specific requirement for heptose in *E. coli* K12. Similar conclusions can be drawn from the work of Schmidt & Lüderitz (1969) with *S. minnesota*.

The proposed location of the receptors in the LPS is summarized in Fig. 4.

The precise structural requirements for a receptor may not, of course, be a single sugar residue; more than one polysaccharide chain may be involved, linkages are probably important (see Prehm *et al.*, 1976a) and other cell surface components may play a role in reception or penetration.

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