Rough mutants of *Salmonella typhimurium* with defects in the heptose region of the lipopolysaccharide core[1]

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Six rough mutants of *Salmonella typhimurium* LT2 with defects in the heptose region of the lipopolysaccharide were isolated and analyzed for phage sensitivity, for sugar composition and serological specificity of the lipopolysaccharide, and for genetic properties. Three of the mutants were of chemotype Rd_2, with mutations presumably in the *rfaF* gene. Three mutants of chemotype Re represent mutants in at least two separate genes, one perhaps *rfaE* and the other a previously undescribed gene *rfaC*, which maps in the cluster of *rfa* genes at 116 min on the linkage map.

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On a isolé six mutants rugueux de *Salmonella typhimurium* LT2 qui ont des défauts dans la région heptose du lipopolysaccharide. Chez ces mutants on a étudié la sensibilité aux phages, la composition en sucres en rapport avec la spécificité sérologique du lipopolysaccharide et les propriétés génétiques. Trois de ces mutants sont du chimiotype Rd_2, les mutations étant présumément sur le gène *rfaF*. Trois mutants de chimiotype Re sont des mutants d'au moins deux gènes distincts, l'un étant peut-être *rfaE* et l'autre *rfaC*, gène non-décrit antérieurement, qui se situe dans le groupement des gènes *rfa* à 116 min sur la carte de liaisons.
[Traduit par le journal]

Introduction

The lipopolysaccharide (LPS) component of the cell wall of gram-negative bacteria, which is the O (somatic) antigen, has been analyzed by chemical (9, 10, 11, 14) and genetic (13, 23) methods (Fig. 1). Mutants in many of the genes that control the synthesis of the O side chains and outer region of the LPS core have been isolated. However, genes involved in the synthesis of the inner region of the LPS core are less well-known, and no genes for synthesis of the lipid A region are known, though the chemical composition of these regions has been determined (9, 12). Mutants with defects in the LPS core may have a defect in a synthetase enzyme, for example, uridine diphosphate (UDP) galactose-epimerase, needed for synthesis of UDP galactose, and so be unable to make the precursor of a specific sugar which is required for insertion in the LPS (3, 4), or may have a defect in a transferase enzyme, such as a glucosyl transferase, and hence be unable to effect transfer of the sugar to a specific site on the LPS (14).

Four genes concerned with the L-glycero-D-mannoheptose units of the LPS core have been described. Mutants of chemotype Rd_2, which lack the distal but not the proximal heptose unit in their LPS (Fig. 1), are due to a mutation in *rfaF*, which is suspected to control a heptose-II transferase (12, 23, 26). A second type is transketolase-deficient and produces Re (heptose-deficient) LPS, indicating that sedoheptulose-7-phosphate is a precursor of the L-glycero-D-mannoheptose (2). The LPS of a third type, *rfaD*, contains a subnormal amount of heptose, including some D-glycero-D-mannoheptose, in addition to some heptose of the ordinary, L-glycero-D-mannoheptose type; this suggests that the

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D-isomer is synthesized first, probably as a nucleotide, then epimerized at position 6 to yield the L-isomer (7). The LPS of the fourth type, *rfaE*, lacks both heptose units (12, 26). Two of the above four genes, *rfaF* and *rfaD*, respectively controlling transferase and synthetase functions, map in the *rfa* gene cluster at 116 min on the linkage map of *Salmonella typhimurium*; the map position of the gene for the transketolase function is unknown. The *rfaE* gene, whose function, synthetase or transferase, is unknown, maps outside the *rfa* gene cluster, around 100 min on the linkage map (6, 7, 17, 21, 26).

This paper reports the isolation, phage sensitivity, LPS sugar analysis and serological character, and genetics of several mutants of *S. typhimurium* LT2 which have lesions in the heptose region of the LPS. Mutants of chemotype Rd_2, inferred to be mutated in the gene *rfaF*, and of chemotype Re (heptose-deficient), with mutations in at least two separate genes, one perhaps *rfaE* and the other a previously undescribed gene, *rfaC*, are described.

Materials and Methods

Strains

The bacterial strains are from the *Salmonella* Genetic Stock Centre, University of Calgary, and the collection of Dr. B. A. D. Stocker (Table 1). The mutants were isolated from a subline of SU418 which is *S. typhimurium* LT2 *proA26* (P22)$^+$ HfrB2 (20); SU418 had been selected for rapid fermentation of trehalose, and then reselected for rapid fermentation of cellobiose, to yield the subline designated PG189 (P. Gemski, personal communication). The rough mutants reported here originally had the HfrB2 chromosome transfer properties of their parent SU418, but during storage they have lost the F-factor, as shown by their infertility and insensitivity to the male-specific phages MS2 and M13.

Bacteriophages

The methods of testing bacterial strains for sensitivity to lysis by bacteriophages have been described (26). For the isolation of bacteriophage-resistant mutants, bacterial cells from a broth culture were flooded onto a plate of nutrient agar, and phage suspension (10^8 pfu/ml) was spread on top; after incubation resistant colonies were picked and purified. The bacteriophages were from the collection of Dr. B. A. D. Stocker.

Media

Difco nutrient broth was used for routine growth of cells. Modified Davis' minimal medium (MM) (20) with 0.2% glucose as carbon source was used as the defined medium; 20 μg/ml of L-amino acids or 2 μg/ml of vitamins were added as supplements where needed. Bile salts sensitivity was tested with 0.4% (w/v) sodium deoxycholate (Sigma) in Difco antibiotic broth No. 3 plus 1.5% agar (ABA). Single enriched medium (SE), used in transduction, is MM to which 1/80 Difco nutrient broth was added. The complete medium used for routine tests and for testing phage sensitivity was Oxoid blood agar base (code CM 55).

Transduction

P22-mediated transduction (18) and induction of P22-lysogens (21) have been described.

Growth of Cells for LPS Extraction

Cells were grown (in Calgary) in 10-liter batches at 37C in a New Brunswick Microferm, using Davis' minimal medium plus 0.1% glucose and 180 μg/ml L-proline. Additional crops of some strains were grown in Freiburg as described before (16). In all cases in which the strain had the *galE* mutation derived from SA22, the bacteria were

TABLE 1

Strains of *Salmonella typhimurium* used in this study[a]

Stock No.	Source	Genotype
SU418[b]	SGSC	*proA26* HfrB2 (P22)$^+$
PG189	SU418	*proA26 tre*$^+$ *clb*$^+$ HfrB2 (P22)$^+$
SL1165[c]	PG189	*proA26 tre*$^+$ *clb*$^+$ *rfa-629* (P22)$^+$ F−
SL1166[c]	PG189	*proA26 tre*$^+$ *clb*$^+$ *rfaF624* (P22)$^+$ F−
SA22[c]	PG189	*proA26 tre*$^+$ *clb*$^+$ *gal-446* (P22)$^+$ F−
SA33[c]	SA22	*proA26 tre*$^+$ *clb*$^+$ *gal-446 rfa-630* (P22)$^+$ F−
SA35[c]	SA22	*proA26 tre*$^+$ *clb*$^+$ *gal-446 rfa-632* (P22)$^+$ F−
SA40[c]	SA22	*proA26 tre*$^+$ *clb*$^+$ *gal-446 rfaF637* (P22)$^+$ F−
SA42[c]	SA22	*proA26 tre*$^+$ *clb*$^+$ *gal-446 rfaF639* (P22)$^+$ F−
SA1356	—[d]	*rfa-630* (P22)$^+$ F−
SL1092	Stocker	*hisD27 trpA8 xyl-412 gal-437 H1-a H2-e,n,x* (Col E1-30) F−
SA1438	SGSC	*pyrE125* F−

[a]SGSC is the *Salmonella* Genetic Stock Centre maintained at the University of Calgary. Many of these stocks came from the late Dr. M. Demerec.

[b]The original HfrB2 isolate (20) is lysogenic for a phage which produced immunity to P22, and which is effective in general transduction; this phage is referred to as P22. Its source is unknown.

[c]These lines originally had the chromosome transfer properties of HfrB2, but during storage they have lost the F-factor.

[d]P22 phage induced by UV from SA33 was used to transduce *pyrE125*; the PyrE$^+$ Rfa transductant was determined, on the basis of phage sensitivity, to be *rfa-630*. This recombinant is lysogenic for the phage from SA33.

grown in medium containing 1% galactose plus 1% glucose, to permit production of smooth LPS, instead of the Rc (galactose-deficient) form.

LPS Extraction

The cells were extracted with phenol–water (PW) (25), or with phenol – chloroform – petroleum ether (PCP) (5); in some cases the residue from PCP extraction was extracted with PW (R-PW). The LPS was partially purified by ultracentrifugation.

Analytical Methods

Estimation of sugars by colorimetric methods or by gas–liquid chromatography of the alditol acetates and paper or thin-layer chromatography were performed as described before (7). The passive hemagglutination inhibition test has also been described (1).

Results

Isolation of rfa "Inner Core" Mutants from a Smooth Strain

Inability to grow in the presence of surface-active agents such as bile salts was observed in mutants with defects in the heptose region of the LPS (26). To detect further mutants with defects of this type, cells of the smooth strain PG189 were treated with the phage Felix O (FO) (also called "O1") and 26 spontaneous resistant mutants were isolated. These mutants were tested for sensitivity to phages which are S-specific (lyse only smooth strains), S- or R- specific (lyse smooth or rough strains), or R-specific (lyse only rough strains), for sensitivity to sodium deoxycholate, and for galactose fermentation (26) (Table 2). Of the 26 mutants, 24 were unaffected by deoxycholate; 10 of these 24 were sensitive to phage C21 and other rough-specific phages (pattern Epi-1 or Epi-2, ref. 26) and unable to utilize galactose, and are therefore inferred to be deficient of UDP galactose-epimerase. The other 14 were resistant to C21 but sensitive to several rough-specific phages (pattern R-res-1 or R-res-2, ref. 26) and retained ability to utilize galactose; from these properties they are inferred to be *rfa* mutants with defects in the outer part of the LPS core. (See Table 2, SA17 and SA20 for typical mutants).

Two of the 26 FO-resistant mutants, SL1165 and SL1166, were sensitive to deoxycholate and to the rough-specific phages Br60 and Ffm, but not to C21. Transduction crosses with these strains were previously reported (21); their LPS character is described below. None of the rough mutants were sensitive to P221.*c2*, as expected for rough mutants of a parent strain lysogenic

TABLE 2

Sensitivity of mutants of *S. typhimurium* to phages and bile salts

Strain No.	Source	Lysis by phage[b] O-specific[a] 9NA	O or R FO	R-specific 6SR	Br2	Ffm	Br60	C21	Growth on DOC[c]	Galactose utilization[d]	Sensitivity pattern
PG189 *rfa*$^+$	SU418	+	+	−	−	−	−	−	+	+	Smooth
SA17 *rfa-623*	PG189	−	−	−	+	+	+	−	+	+	R-res-1
SA20 *rfa-625*	PG189	−	−	−	−	+	+	−	+	+	R-res-2
SL1166 *rfaF624*	PG189	−	−	−	−	+	+	−	−	+	R-res-2
SL1165 *rfa-629*	PG189	−	−	−	−	+	+	−	−	+	R-res-2
SA22 *gal-446*	PG189	−	−	−	−	+	+	+	+	−	Epi-2
SA33 *rfaC630*	SA22	−	−	−	−	±	+	−	−	−	Reduced-R-res-2
SA35 *rfa-632*	SA22	−	−	−	−	±	+	−	−	−	Reduced-R-res-2
SA40 *rfaF637*	SA22	−	−	−	−	+	+	−	−	−	R-res-2
SA42 *rfaF639*	SA22	−	−	−	−	+	+	−	−	−	R-res-2

[a]All the strains shown are lysogenic for P22 (or a related phage) which confers immunity to P22, P22 *h*, and P221 (24).
[b]Phage sensitivity was tested as described in Materials and Methods.
[c]Bile salts sensitivity was tested on NA plus 0.4% sodium deoxycholate (DOC).
[d]Galactose utilization was assayed by growth on MM plus galactose plus proline.

for P22 or a related phage, since P22 lysogeny confers immunity to P221.*c2* (24).

Isolation of rfa "Inner Core" Mutants from a galE Mutant

Some galactose non-fermenting mutants, resulting from lack of UDP-galactose 4-epimerase (3), make galactose-deficient LPS (chemotype Rc), are galactose-sensitive, and show the phage-sensitivity pattern termed Epi-1, which includes sensitivity to phage C21 (26). If the galactose kinase and uridyl transferase enzymes are present in the cell, phenotypic reversion to the smooth form is possible through conversion of galactose from the medium into UDP galactose and its use in making normal LPS. Mutants of chemotypes Rd_1 (*rfaG* and *galU*; glucose-deficient) are sensitive to phage C21 (26; Kuo, MacPhee, and Stocker, unpublished data); therefore C21-resistant mutants of C21-sensitive lines (if they are not smooth revertants) are expected to have "deeper" lesions in the LPS, i.e., to have lesions in the heptose, ketodeoxyoctonate (KDO), or lipid A region. The 10 galactose-negative mutants obtained from PG189 by selection for resistance to phage FO were tested for sensitivity to the R-specific phages C21, Ffm, and Br60, and to phages FO and 9NA, on the following four media: ABA, ABA + 2% galactose, ABA + 2% glucose, ABA + 2% glucose + 2% galactose. Three mutants failed to grow in the presence of galactose unless glucose was also present, and were sensitive to smooth-specific phages and resistant to rough-specific phages, when tested on medium containing glucose plus galactose. These three mutants were inferred to be epimerase mutants, mutated at *galE*. The other seven galactose-negative C21-sensitive mutants may have mutations affecting *galK* or *galT* function, as well as *galE* function, or may be *galU* mutants. From one of the *galE* mutants, SA22, 18 C21-resistant mutants were isolated. The growth of all 18 mutants was inhibited in the presence of 0.4% sodium deoxycholate. Six mutants, SA33 to SA38, were sensitive to phage Br60, and partially sensitive to Ffm, but resistant to all other R-specific phages tested, while 12 mutants, SA39 to SA50, were completely lysed by both Ffm and Br60. The sensitivity patterns of two mutants of each type, SA33, SA35, SA40, and SA42, are shown in Table 2. All 18 C21-resistant mutants of the *galE* derivative of PG189 thus had the phenotype expected for mutants with "deep" LPS defects: two of the mutants partly resistant to Ffm, and two mutants retaining sensitivity to this phage were investigated in respect of LPS character and gene location.

LPS Composition of the Mutants

The chemotype of the LPS extracted by the PCP and (or) PW method from six of these deoxycholate-sensitive mutants is shown in Table 3. LPS extracted from SL1166 by PW contains significant amounts of glucose and galactose, and also of the O-specific sugars mannose, rhamnose, and abequose: in the hemagglutination inhibition (HAI) tests it inhibits most strongly the Rd_2 system. LPS isolated by the PCP method, which preferentially extracts rough rather than smooth LPS, contains abundant KDO and heptose, but only negligible amounts of glucose, galactose, and the O-specific sugars. Therefore SL1166 makes Rd_2 LPS, and also considerable smooth LPS, indicating that it is a "part rough" mutant, presumably with a "leaky" mutation in a gene determining an enzyme for attachment of the distal heptose (Fig. 1). The composition of the LPS from the PW extract of SL1165 resembles that of SL1166, in that it contains O-specific sugars; but in HAI tests it reacts most strongly in the Re system. The PCP-extracted material contains KDO, but no other core sugars and no O-specific sugars. These data indicate that SL1165 is a part-rough Re mutant.

Six of the C21-resistant mutants selected from a *galE* parent strain, SA22, were partly resistant to phage Ffm. PCP extraction of one of these six, SA35 (*rfa-632*), gave LPS containing KDO but no glucose, galactose, or O-specific sugar and of serological character Re, according to HAI tests (Table 3). The type of LPS determined by the *rfa* allele of another mutant partly resistant to Ffm, viz. SA33 (*rfa-630*), was tested by examination of a $pyrE^+$ *rfa-630* clone, SA1356, obtained by cotransduction as described in the next section. PCP extraction of SA1356 cells yielded LPS of type Re like that obtained by PCP extraction of SA35 (Table 3). PW extraction of the residues of PCP-extracted SA35 and SA1356 cells yielded no LPS. Thus both SA35 and SA33 are non-leaky rough mutants making LPS of type Re, i.e. heptose-deficient. Twelve of the strains selected from SA22 were completely

TABLE 3

Sugar composition and serological specificity of lipopolysaccharides

Strain No.	Extraction[a] method	% weight of LPS, per mg of dried bacteria	Amount of sugars, % of total LPS[b]							Chemotype (by HAI test)
			KDO	Hep[c]	Glc	Gal	Man	Rha	Abe	
SL1166	PW	2.0	5.5	6.0	3.8	6.9	5.8	5.4	3.1	Rd_2 part-rough[d]
rfaF624	PCP	0.6	8.0	7.0	0	0	0	0	0	
SL1165	PW	3.7	7.3	9.6	5.2	6.2	5.2	4.6	2.9	Re part-rough
rfa-629	PCP	1.0	15.5	0	0	0	0	0	0	
SA22	PCP	0.0								
gal-446	R-PW	2.9	+	+	+	+	+	+	+	S
SA1356[e]	PCP	6.5	11.2	0	0	0	0	0	0	Re
rfaC630	R-PW	0								
SA35	PCP	4.0	14.1	0	0	0	0	0	0	Re
rfa-632	R-PW	0								
SA40	PW	0.2[f]	12.0	7.0	0	0	0	0	0	Rd_2
rfaF637	PCP	2.5	13.0	8.0	0	0	0	0	0	
SA42	PCP	1.8	8.6	4.5	0	0	0	0	0	Rd_2 part-rough
rfaF639	R-PW	0.7	+	+	+	+	+	+	+	

[a]PW, phenol–water extraction; PCP, phenol – chloroform – petroleum ether extraction; R-PW, phenol–water extraction of the residues of cells after PCP extraction. The PCP method extracts R lipopolysaccharide; the S form is not extracted by this method. The PW method extracts both R and S lipopolysaccharides. S-form lipopolysaccharide remaining on the cells after PCP extraction is isolated by PW extraction.

[b]KDO was determined by colorimetric methods, the other sugars by gas–liquid chromatography (7). In some cases qualitative analysis was performed by thin-layer chromatography; + = present, − = absent. HAI = passive hemagglutination inhibition test, performed according to Beckmann *et al.* (1). Strains SA22, SA35, SA40, and SA42, all of which carry a mutation for inability to ferment galactose, were grown for extraction on a culture medium containing 1% galactose plus 1% glucose.

[c]All detectable heptose was the L-glycero-D-mannoheptose isomer according to gas–liquid chromatography.

[d]Part-rough indicates HAI activity in S (smooth) as well as in the indicated rough system.

[e]SA1356 is a transductional derivative of SA33 (see text).

[f]It is known that PW extraction is sometimes ineffective in isolation of LPS from Re mutants.

sensitive to Ffm. One of them, SA40, was extracted by PW and by PCP. LPS of identical composition were obtained by each method: they contained KDO and heptose, but no traces of glucose, galactose, or O-specific sugars, and inhibited the Rd_2 system in HAI, indicating that this strain has a non-leaky mutation for Rd_2 LPS. Similar results were obtained with the PCP extract of SA42; this strain, however, yielded S form LPS on extraction with phenol–water, indicating an Rd_2 "part rough" chemotype.

Genetic Analysis of rfa Mutants

Transduction crosses with rough mutants are normally not possible with the bacteriophage P22, because these mutants are resistant to P22. However, SU418 (HfrB2) and the *rfa* mutants derived from it are lysogenic for phage P22 (or a similar phage), and transducing phage was obtained by induction of these lysogens. Since transduction studies using P22 and ES18 phage (6, 21) have shown that many *rfa* alleles are located between *cysE* and *pyrE* on the *S. typhimurium* linkage map, auxotrophs for these genes were used as recipients in crosses with the six rough strains as donors, and the transductants were tested for their Rfa phenotype as indicated by sensitivity to phages FO and Ffm, and to sodium deoxycholate. Previously reported results for SL1165 and SL1166 (21) as well as recent results (Table 4) indicate that the *rfa* alleles of the three mutants with LPS of chemotype Rd_2 (or part-rough Rd_2), namely SL1166, SA40, and SA42, are jointly transduced with *cysE* at frequencies of 26% to 46% and with *pyrE* at frequencies of 10% to 13%. Of the mutants with LPS of chemotype Re (or part-rough Re), the *rfa* allele of SA33 was jointly transduced with *cysE* (32%) and *pyrE* (14%), but joint transduction of the *rfa* alleles of SL1165 and SA35 with *cysE* or *pyrE* was not detected.

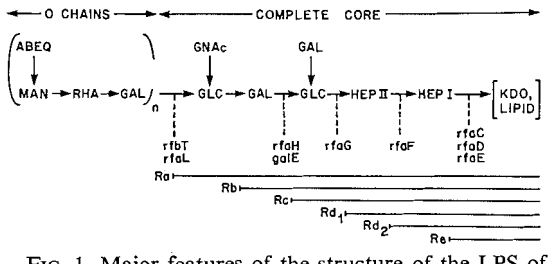

FIG. 1. Major features of the structure of the LPS of *S. typhimurium*, and symbols for the genes known or assumed to form the indicated bonds, or, in the case of *rfaC*, *rfaD*, and *rfaE*, the indicated sugar (see ref. 23). The symbols Ra to Re indicate the components present in LPS of the indicated chemotype (9).

TABLE 4

Joint transduction by phage P22 of *rfa* genes

Donor	Unselected donor gene	Joint transduction with *cysE396*[a]		Joint transduction with *pyrE125*	
SA33	*rfaC630*	(31/97)	32%	(8/58)	14%
SA35	*rfa-632*	(0/58)	0%	(0/51)	0%
SA40	*rfaF637*	(21/46)	46%	(4/39)	10%
SA42	*rfaF639*	(9/35)	26%	(6/50)	12%

[a]The denominator of the fraction indicates the total number of CysE+ transductants tested; the numerator is the number which is Rfa in phenotype, as determined by sensitivity to phages FO, Ffm, and Br60 and to sodium deoxycholate.

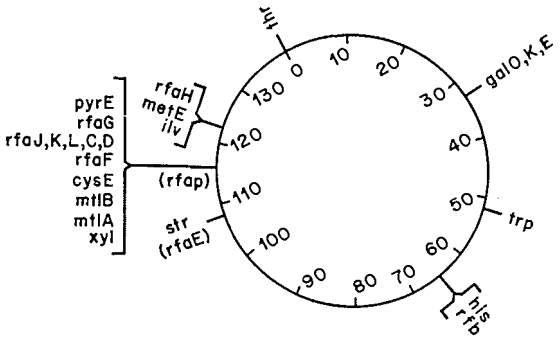

FIG. 2. A partial linkage map of *S. typhimurium*, redrawn from ref. 17. The map is marked in 10-min. intervals, which were determined by interrupted conjugation crosses. The genes shown in arcs represent phage-mediated transduction linkage groups. Genes shown in parentheses are only approximately located. The gene order *pyrE-rfaG-(rfaJ, K, L)-rfaF-cysE* was established by joint transduction studies (6, 21), but the location of *rfaC* and *rfaD* relative to other *rfa* genes is not known.

Discussion

Three of the rough mutants, SA1166 *rfa-624*, SA40 *rfa-637*, and SA42 *rfa-639*, are of chemotype Rd_2 (or part-rough Rd_2) and all three alleles mapped in the *rfa* gene cluster between *cysE* and *pyrE*. Previously three mutants of *S. typhimurium* of the same chemotype, all of which are jointly transduced with *cysE* and *pyrE* at about the same frequencies as the mutants reported here (6, 26), were designated *rfaF*. The enzymatic lesion has not been determined for any of these six alleles, but the presence of heptose I and the absence of heptose II on each LPS is consistent with a defect in the heptose II transferase, but not with a mutation in a gene for synthesis of heptose units. Because of similarity of genetic location, and the inference that the loss of only a single biochemical function can explain this defect, these six alleles are designated *rfaF*.

The remaining three strains produced LPS of chemotype Re (or part-rough Re). The *rfa* alleles of two of these strains, SL1165 *rfa-629*, and SA35 *rfa-632*, are not linked by transduction to *cysE* or *pyrE*, though conjugation crosses indicate that the *rfa* allele of SA1165 is located in the *xyl* region of the chromosome. Thus these two mutants may resemble SL1102 *rfaE543* which produces LPS of chemotype Re with the mutation located in the 95- to 105-min segment of the linkage map, fairly close to *xyl* (6, 26). The mutant locus of SL1102 was designated *rfaE*, and the mutants of SL1165 and SA35 may be in the same gene. As judged by tests on a transductional derivative, SA1356, mutant SA33 *rfa-630* produces LPS of chemotype Re, but the mutation is linked by transduction to *cysE* and *pyrE*; this mutation therefore affects a locus concerned with formation of the heptose I unit, distinct from locus *rfaE*, which maps outside the *cysE–pyrE* segment (6). A biosynthetic pathway for the presumed nucleotide form of L-glycero-D-mannoheptose, starting from sedoheptulose-7-phosphate and requiring an isomerase, a mutase, a synthase, and an epimerase has been postulated, and mutants in transketolase have been isolated and studied (2). A part-rough mutant with LPS containing some D-glycero-D-mannoheptose as well as some heptose of the usual L-glycero-D-mannoheptose form was designated *rfaD* and inferred to have a defect in the postulated epimerase (7). The LPS of this leaky *rfaD* mutant contained some heptose I units made up of the D-glycero-D-mannoisomer, which shows that the enzyme for formation of the heptose I unit can transfer the unusual isomer,

though less efficiently than the normal form. The LPS of the transductional derivative of the non-leaky Re mutant under discussion, SA33, did not contain any detectable amount of heptose, of either isomeric form (Table 3). It is therefore likely that its mutation, *rfa-630*, affects a gene other than *rfaD*, which is believed to specify the postulated epimerase on the heptose biosynthetic pathway: we therefore designate this mutation *rfaC630*. The four mutations with Re chemotype, three described in this paper and SL1102 *rfaE543* (6, 26) thus represent mutations in at least two different genetic locations. They may have deficiencies of any of the other four postulated functions, isomerase, mutase, synthase, and heptose I transferase. A mutant of chemotype Re in *E. coli* 08:K27 is due to a mutation which appears to be located in the same region of the chromosome as *rfaE* in *S. typhimurium* (22).

In the selection of C21-resistant mutants from the galactose-epimerase strain, strains with defects in the KDO or lipid A region of the LPS, if they occur, should be isolated. The fact that they were not discovered suggests that such mutants may be unable to form colonies, at least on nutrient agar at 37C. A mutant unable to synthesize the KDO component of the LPS was dependent for growth on a supply of its precursor, D-arabinose-5-phosphate, further indicating that some components of the LPS are essential for cell growth (15). Mutants with LPS which is deficient in components of the core have altered physiology; the strains reported in this paper have enhanced sensitivity to most antibiotics and to lysozyme, as shown in an accompanying paper (19). They also show enhanced release of a periplasmic enzyme, alkaline phosphatase (8).

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