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Formation of the K30 (Group I) Capsule in *Escherichia coli* O9:K30 Does Not Require Attachment to Lipopolysaccharide Lipid A-Core

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***Escherichia coli* K antigens (capsular polysaccharides) are divided into two broad classes, designated groups I and II, on the basis of a number of chemical, physical, and genetic criteria. Group I K antigens can be further subdivided on the basis of the absence (group IA) or presence (group IB) of amino sugars in the repeating unit of the K antigen. One criterion proposed for inclusion in group I is covalent linkage of the capsular polysaccharide to the lipid A-core of lipopolysaccharide (LPS). *E. coli* O9:K30 is a strain with a representative group IA K antigen. This organism synthesizes an LPS-associated low-molecular-weight form of K30 antigen which is called K_{LPS}. To determine the involvement of LPS lipid A-core in expression of the K30 capsular polysaccharide, *E. coli* K30/K-12 hybrid strains were constructed with mutations in the *E. coli* K-12 *rfa* locus, responsible for the biosynthesis of the LPS core oligosaccharide. These strains lack K_{LPS}, indicating that a full-length core is required for K_{LPS} expression. However, formation of a K30 capsule was unaffected by *rfa* defects, indicating that attachment to lipid A-core is not an obligatory step for either export of high-molecular-weight capsular polysaccharide or maintenance of the capsular structure on the cell surface. Silver-stained tricine-sodium dodecyl sulfate–polyacrylamide gel electrophoresis profiles of lipopolysaccharides from other *E. coli* K serotypes showed that all strains with group IB K antigens expressed some K_{LPS}. In contrast, some strains with group IA K antigens appear to lack K_{LPS}. Consequently, although association of group I K antigens with lipid A-core is common, it is not a universal marker for inclusion in group I.**

There are over 70 different capsular polysaccharides (CPSs) or K antigens in *Escherichia coli* (45), and the structures of many of these polysaccharides are known (5). *E. coli* K antigens are divided into two groups (I and II) on the basis of chemical, physical, and genetic criteria (18). The thermolabile group II K antigens resemble CPSs of *Neisseria meningitidis* and *Haemophilus influenzae* (18). The thermostable group I K antigens are further subdivided on the basis of the absence (group IA) or presence (group IB) of amino sugars in the polysaccharide repeating unit (20, 22). The structures of the *E. coli* group IA K antigens resemble CPSs found in *Klebsiella* spp. (20, 22). Research in this laboratory has focused on the synthesis and expression of the *E. coli* K30 CPS as a prototype of group IA. The structure of the K30 CPS is shown in Fig. 1A.

Several *E. coli* group II K antigens contain phosphatidic acid residues at the reducing terminus (12, 52). The thermolability of *E. coli* group II K antigens at a pH of <6 reflects an acid-labile linkage to phosphatidic acid. The lipid substituents are thought to anchor the group II K antigens to the cell surface (18). Similar phospholipid substituents are found in the CPSs of *N. meningitidis* (12) and *H. influenzae* (6, 33).

The nature of the linkage of group I CPSs to the cell surface is not well characterized, although association with lipid A-core is one of the criteria used to classify group I K antigens (18). Recently, Jann et al. (19) showed that a portion of the group IB K40 polysaccharide is covalently linked to the core oligosaccharide of the lipopolysaccharide (LPS) in *E. coli* O8:K40. Consequently, *E. coli* O8:K40 attaches two polysaccharide antigens to lipid A-core: the neutral polymannose O8 *O*-polysaccharide and the acidic K40 antigen. *E. coli* O9:K30 also appears to express a portion of the K30 antigen in a form attached to the LPS core (16, 58), although the degree of polymerization of K30 antigen attached to LPS core is much less than that in *E. coli* O8:K40. Nothing is known about the mode of cell surface association of the high-molecular-weight K30 CPS of *E. coli* O9:K30.

To determine whether high-molecular-weight CPS is linked to the cell surface via lipid A-core or whether linkage to lipid A-core is an obligatory intermediate in the biosynthesis of high-molecular-weight K30 CPS, we have constructed *E. coli* K-12–*E. coli* K30 hybrids (K-12/K30) containing defined mutations in the K-12 *rfa* (LPS core biosynthesis) genes. The results demonstrate that the K30 antigen is surface expressed by two independent pathways. K30 oligosaccharides are attached to the cell surface by lipid A-core. In contrast, high-molecular-weight K30 CPS, which forms the capsular structure, is assembled and exported by an LPS-independent pathway.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are summarized in Table 1. Cells were routinely cultured in Luria-Bertani broth (42). M9 medium (GIBCO) was the defined medium; carbon sources (0.2%), amino acids (40 μg/ml), thiamine and nicotinamide (1 μg/ml), and uracil (40 μg/ml) were added where appropriate. For solid media, Bacto agar was added to a final concentration of 1.5%. Antibiotics were added when required at the following concentrations: chloramphenicol, 25 μg/ml; streptomycin, 200

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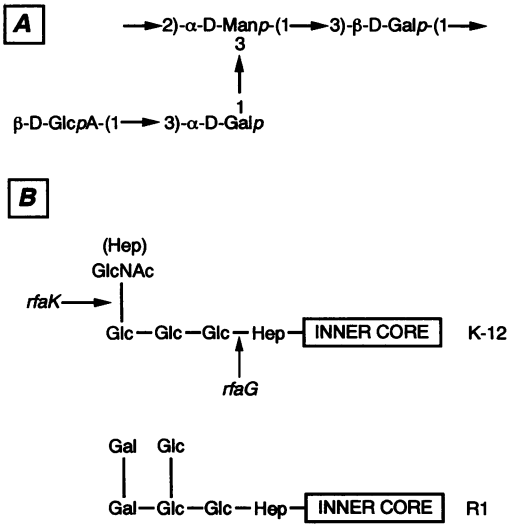

FIG. 1. Structures of (A) the K30 CPS (2) and (B) the hexose region of the K-12 (15, 23) and R1 (23) LPS core oligosaccharides from *E. coli*. The sugar additions to the K-12 core affected by the *rfaK* and Δ*rfaGPSBI* mutations are shown.

μg/ml; kanamycin, 30 μg/ml; novobiocin, 80 μg/ml. Incubation was at 37°C.

Determination of O9 and K30 expression. Bacteriophage sensitivity patterns were used to ascertain the LPS chemotype and the expression of K30 CPS. Sensitivity patterns were determined with Luria-Bertani agar by the method of Wilkinson et al. (60); titers of the applied bacteriophages were adjusted to 10^8 PFU/ml, and bacteriophage sensitivities were read after 6 to 8 h of incubation. The coliphages K30 (57) and O9-1 (40) have been described elsewhere; these bacteriophages are specific for K30 CPS and O9 LPS, respectively. Phages C21, Ffm (60), and P1*vir* (54) were obtained from K. E. Sanderson (University of Calgary, Alberta, Canada). The presence of K30 antigen was also determined by slide agglutination with the K30-capsule-specific monoclonal antibody (MAb) 4-15A (16). Acapsular, coliphage K30-resistant mutants were isolated as described previously (58). Plasmid pWQ607 was used to test for the complete LPS core oligosaccharide in *E. coli* derivatives. pWQ607 is a 45-kb cosmid derivative of the vector pVK102 (30), obtained from a chromosomal gene bank of *E. coli* O9:K30 strain E69. Construction of the gene bank was described previously (25). This plasmid contains the *gnd-rfb*$_{O9}$ region (24).

Isolation of purified LPS and CPS. LPS and CPS (total cell surface polysaccharide) were isolated with hot aqueous phenol by a modification of the method of Johnson and Perry (26); after dialysis, the polysaccharide preparations were treated with additional DNase, RNase, and proteinase K digestion steps. LPS and CPS were partially purified by differential centrifugation at 106,000 × *g* for 16 h; the CPS fraction was the supernatant of the first centrifugation. To prepare the LPS fractions, the pellets from the ultracentrifugation step were dispersed by sonication in water and washed twice by repeated ultracentrifugation steps.

Further purification of CPS was effected by gel filtration through a Sephacryl S300 column (90 by 1.6 cm; Pharmacia) equilibrated with DOC buffer (3% [wt/vol] sodium deoxycholate, 0.2 M NaCl, 5 mM disodium EDTA, and 10 mM Tris-HCl [pH 8.0]) (37). Sephacryl S300 has a dextran exclusion limit of 100,000 Da, according to the manufacturer's specifications. Fractions (2.5 ml) were collected at a flow rate of 0.15 ml/min and assayed for total hexoses by the acid-phenol method of Dubois et al. (4). Detergent was removed from pooled column fractions by extensive dialysis, first against column buffer not containing sodium deoxycholate and then against distilled water. Polysaccharides were recovered by lyophilization.

Gel electrophoresis of LPS. LPS from proteinase K-digested whole-cell lysates was isolated from stationary phase cultures as described by Hitchcock and Brown (14). Electrophoresis was through 16.5% polyacrylamide gels with the Tricine-sodium dodecyl sulfate (SDS) buffer system of Lesse et al. (35). LPS gels were silver stained as described previously (56).

Determination of sugar composition. Sugar composition was determined after mild acid hydrolysis and trifluoroacetic acid hydrolysis of polysaccharides by methods described previously (3).

Capsule stabilization and electron microscopy. The capsule morphology of *E. coli* O9:K30 and its derivatives was examined by electron microscopy, after antibody stabilization of the capsule, by methods described previously (59). Briefly, cells from an overnight Luria-Bertani broth culture were washed with 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 6.8) and resuspended in complement-inactivated MAb 4-15A (16) ascites fluid. The samples were incubated at 4°C for 1 h. Antibody-stabilized cells were then washed once in HEPES buffer, glutaraldehyde fixed, and stained with ruthenium red. Fixed and stained cells were immobilized in 2% Noble agar, dehydrated, stained with osmium tetroxide, and embedded in Epon 812. Sections were poststained with uranyl acetate and lead citrate prior to examination with a Philips EM300 electron microscope operating at 60 kV.

RESULTS AND DISCUSSION

Core type of *E. coli* O9:K30. Five different LPS core structures are known in *E. coli* (23). *E. coli* O9:K30 is inferred to have an R1 core on the basis of phage sensitivity patterns and the sugar composition of the LPS of *E. coli* CWG57. CWG57 is an O^-K^- derivative of CWG44 obtained by selecting for resistance to the K30-specific coliphage K30. The absence of K30 antigen on the cell surface of CWG57 was confirmed by electron microscopy (38), sugar composition of purified LPS (3), and the absence of agglutination with MAb 4-15A (Table 2), a MAb specific for K30 antigen (16). A recombinant plasmid, pWQ607, containing the *rfb*$_{O9}$ gene cluster was used to transform CWG57. The transformants were susceptible to bacteriophage O9-1, which is specific for strains expressing the O9 antigen. In SDS-polyacrylamide gel electrophoresis (SDS-PAGE), CWG57(pWQ607) produced a ladder pattern with smooth LPS identical to that of authentic O9 strains (38). The LPS core of CWG57 is therefore complete, because any core deficiency would prevent it from acting as an acceptor for attachment of the cognate O9 antigen when the cloned *rfb*$_{O9}$ cluster is present. CWG57 LPS does not contain *N*-acetylglucosamine (3), indicating that the core is probably either an R1 or R4 type (23). Some *E. coli* K-12 strains also lack *N*-acetylglucosamine in the hexose region (15). However, because CWG57 is resistant to phage U3 (38) and sensitive to phage C21 (Table 2), it does not have a K-12 type core. R1 and R4 type cores can be distinguished by the relative ratios of glucose and galactose. Because the CWG57 core has a molar excess of glucose (3), it has an R1 type core (Fig. 1). The R1 core is

TABLE 1. *E. coli* strains used in this study

Strain	Genotype or derivation	Serotype[a]	Source or reference
E69	Prototroph	O9:K30:H12 (IA)	I. Ørskov
CWG28	E69 derivative; cps_{K30} mutant *his trp lac rpsL*	O9:K$^-$	58
CWG44	E69 derivative; rfb_{O9} mutant *his trp lac rpsL*	O$^-$:K30	40
CWG57	CWG44 derivative; rfb_{O9} mutant cps_{K30} mutant *his trp lac rpsL*	O$^-$:K$^-$	3
CS2057	*thr-1 leuB6 lacY1 supE44 rfbD1 thi-1 ara-14 galK2 xyl-5 mtl-1 mgl-51 proA2 hisG4 kdgK51 argE3 rac* mutant *rpsL31 non trpE* λ mutant Δ*rfaGBPI*::Cml^r *cps-5*::Tn*10*	O$^-$:K$^-$	C. A. Schnaitman[b]
CS2529	*thr-1 leuB6 lacY1 supE44 rfbD1 thi-1 ara-14 galK2 xyl-5 mtl-1 mgl-51 proA2 hisG4 kdgK51 argE3 rac* mutant *rpsL31 non trpE* λ mutant *rfaK2*::ΩKan^r	O$^-$:K$^-$	29
KL800	Hfr; PO131 of HfrPK3	O$^-$:K$^-$	36
PR3339	KL800 but Δ*rfaGBPI*::Cml	O$^-$:K$^-$	This study[c]
PR3358	CWG44 but Δ*rfaGBPI*::Cml	O$^-$:K30	This study[d]
PR3954	KL800 but *rfaK2*::ΩKan^r	O$^-$:K$^-$	This study[e]
PR3957	CWG44 but *rfaK2*::ΩKan^r	O$^-$:K30	This study[f]
G3404-41		O8:K8:H$^-$ (IB)	F. Ørskov
2667		O9:K9:H$^-$ (IB)	B. Jann
2146		O9:K26:H$^-$ (IA)	B. Jann
E56b		O8:K27:H$^-$ (IA)	F. Ørskov
K14a		O9ab:K28:H$^-$ (IA)	F. Ørskov
Bi161-42		O9:K29:H$^-$ (IA)	F. Ørskov
Su3973-41		O9:K31:H$^-$ (IA)	F. Ørskov
E75		O9:K34:H$^-$ (IA)	F. Ørskov
2150		O9:K37:H$^-$ (IA)	B. Jann
2151		O9:K39:H9 (IA)	B. Jann
2775		O8:K40:H9 (IB)	B. Jann
2176		O8:K41:H11 (IB)	B. Jann
A295b		O8:K42:H$^-$ (IA)	F. Ørskov
2178		O8:K43:H11 (IA)	B. Jann
2167		O8:K45:H$^-$ (IB)	F. Ørskov
2181		O8:K46:H4 (IB)	B. Jann
2182		O8:K47:H2 (IB)	B. Jann
2183		O8:K48:H9 (IB)	B. Jann
2184		O8:K49:H21 (IB)	B. Jann
2185		O8:K50:H9 (IB)	B. Jann
N24c		O9:K55:H$^-$ (IA)	F. Ørskov
D227		O8:K87:H19 (IB)	F. Ørskov

[a] The group designation for the K antigen (IA or IB) in parentheses is from reference 25.
[b] The construction of Δ*rfaGPSBI*::Cml^r is described in reference 47.
[c] KL800 was transduced to Cml^r with P1*vir* grown on CS2057.
[d] PR3339 was mated with CWG44 with selection for Cml^r; Str^r was used for counter selection.
[e] KL800 was transduced to Kan^r with P1*vir* grown on CS2529.
[f] PR3954 was mated with CWG44 with selection for Kan^r; Str^r was used for counter selection.

found in other *E. coli* strains with group IA and IB K antigens (50). Recent studies with clinical *E. coli* isolates (8) showed the association of the O8 antigen with the R1 core type. Group I K antigens are found primarily in strains expressing the O8 and O9 antigens (18, 45).

TABLE 2. Phenotypes of *E. coli* strains

Strain	Sensitivity to bacteriophage:			Agglutination in MAb 4-15A[a]	Sensitivity to:	
	K30	C21	Ffm		Novobiocin[b]	Bile salts[c]
E69	+	−	−	+	−	−
CWG44	+	−	−	+	−	−
CWG57	−	+	+	−	−	−
PR3358	+	−	−	+	+	+
CS2057	−	+	+	−	+	+
PR3957	+	−	−	+	−	−
CS2529	−	+	+	−	−	−

[a] Specific for the K30 antigen (16).
[b] Novobiocin (80 μg/ml) was incorporated into Luria-Bertani plates.
[c] Determined by the ability to grow on MacConkey plates.

A complete LPS core is required for K_{LPS} expression. The K30 antigen in *E. coli* O9:K30 is expressed in at least two forms: a high-molecular-weight, capsular form and a low-molecular-weight, LPS-associated form (16, 58) called K_{LPS}. The K_{LPS} designation is used to indicate a putative association with lipid A-core of LPS but to distinguish it from O antigen. A similar designation has been used to distinguish between LPS-linked and phosphatidylglycerol-linked forms of the enterobacterial common antigen (ECA) (31). K_{LPS} of *E. coli* O9:K30:H12 copurifies with LPS in phenol-water extracts followed by column chromatography and migrates in SDS-PAGE gels like a semirough LPS species (16, 58) (Fig. 2, lanes 1 and 2). K_{LPS} is found in all *E. coli* O9:K30 strains expressing K30 antigen and is absent in acapsular strains like CWG57 (Fig. 2, lane 3). Because the *E. coli* K-12 core can act as an acceptor for K30 antigen (34), we constructed hybrid *E. coli* K30/K-12 strains containing defined *rfa* (LPS core biosynthesis) mutations of *E. coli* K-12 to confirm association of K_{LPS} with lipid A-core and to assess the requirement for linkage of K antigen to lipid A-core for expression of high-molecular-weight CPS on the cell surface.

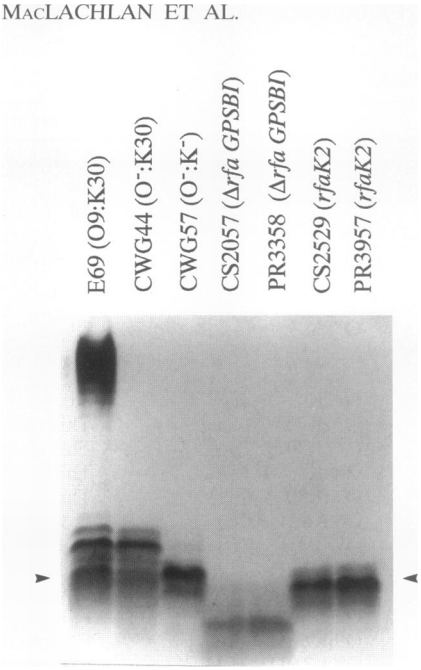

FIG. 2. Tricine–SDS-PAGE of LPS from *E. coli* strains. Lanes 1 to 3 show the LPS from strains E69 (O9:K30), CWG44 (O^-:K30), and CWG57 (O^-:K^-), respectively. CWG57 provides a control for the migration of the complete R1 core (indicated by the arrow). Lanes 5 and 7 contain LPS from the *rfaGPSBI* (PR3358) and *rfaK2* (PR3957) mutants of *E. coli* CWG44, respectively. The LPS from the K-12 strains CS2057 (*rfaGPSBI* [lane 4]) and PR3358 (*rfaK2* [lane 6]) are shown for comparison. The samples were prepared from SDS-proteinase K-digested whole cells (14).

E. coli CS2057 is a K-12 strain with an *rfaGPSBI* deletion in which an *Nco*I fragment of the *rfa* cluster has been replaced with a Cml^r cassette (called Δ*rfa1* in reference 47). CS2057 has a deep rough (Rd_1 P^-) LPS in which the hexose region is absent (Fig. 1B). Because the *E. coli* O9:K30 strain E69 and its derivatives are resistant to bacteriophage P1 (38), the *rfaGPSBI* mutation was transferred to CWG44 by Hfr-mediated conjugation, with PR3339 as the donor (Table 1); selection was for Cml^r. Several independent transconjugants were examined; because they were indistinguishable in their LPS profiles, and in tests of phage, antibiotic and bile salts sensitivities, only one (*E. coli* PR3358) was chosen for detailed study.

E. coli PR3358 had the expected deep rough phenotype (Table 2). It was sensitive to novobiocin and bile salts, and the LPS profile (Fig. 2, lane 5) showed a truncated core that comigrated with the Rd_1 P^- LPS of CS2057 (lane 4). No K_{LPS} band was seen as predicted if K_{LPS} is linked to full-length LPS core.

Deep rough mutations severely perturb outer membrane architecture. Outer membrane permeability is altered, there are changes in outer membrane protein composition, and the normal asymmetry of the outer membrane is reduced (reviewed in reference 44). To ensure that loss of K_{LPS} was not due to a secondary effect of the deep rough phenotype, the *rfaK2* allele of *E. coli* K-12 CS2529 was transferred to CWG44 by the strategy described above to yield PR3957 (Table 1). In *Salmonella enterica* serovar Typhimurium, the *rfaK* gene product is thought to be a hexose transferase which adds an α-1,2-linked *N*-acetylglucosamine residue to the distal end of the core (60). By analogy, the *E. coli* K-12 *rfaK* product is also thought to be an *N*-acetylglucosamine transferase (29) but the site of attachment has not been unambiguously assigned. Early structural data suggested that the *N*-acetylglucosamine residue was a nonstoichiometric substituent at the distal end of the core (23). More recent structural data did not conclusively identify the linkage site for the GlcNAc residue (15), and it has been suggested that *rfaK* may even modify the heptose region of the inner core (29).

E. coli PR3957 produced LPS which comigrated with CS2529 LPS in SDS-PAGE, and no K_{LPS} was seen (Fig. 2, lanes 6 and 7). Again the results confirm the requirement for a complete LPS core for expression of K30 antigen in its low-molecular-weight form. Although the precise role of *rfaK* in LPS core assembly is not known, it is interesting that the *rfaK* requirement for K_{LPS} expression parallels the *rfaK* requirement for attachment of O antigen to the K-12 core (29).

In addition to the defined mutations described above, a series of mutants with truncated LPS cores were isolated after ethyl methanesulfonate mutagenesis of *E. coli* CWG44. No K_{LPS} was seen with any mutant showing a truncated core (38).

Surface expression of high-molecular-weight K30 CPS and capsule formation does not require an intact LPS core. Although K_{LPS} is absent in PR3358 and PR3957, these strains remain sensitive to phage K30 and are agglutinated by MAb 4-15A (Table 2), indicating that the K30 antigen is still expressed on the cell surface. Furthermore, the amount of K30 CPS produced is sufficient to mask receptors for the rough specific phages C21 and Ffm, which infect O^-K^- mutants such as CWG57 (Table 2).

Group I K antigens are typically large with molecular masses in excess of 100,000 Da (18) and are not usually resolved into a well-defined ladder in SDS-PAGE gels, a pattern typical of other surface polysaccharides such as ECA (31), O antigen containing LPS (10, 14, 43, 46), group II CPSs (48), or the low-molecular-weight form of the group I K40 CPS (19). Presumably this is due to the large size of the polymer, which excludes it from the gel matrix. To determine whether the polysaccharide produced by K30 *rfa* mutants was high molecular weight, we used two methods: gel filtration and electron microscopy.

CPSs from *E. coli* CWG44 and PR3358 were extracted with hot aqueous phenol, partially purified by ultracentrifugation, and then purified by S300 Sephacryl gel filtration in deoxycholate buffer. Both strains produced high-molecular-weight polysaccharide which eluted at the void volume (38), indicating an apparent molecular mass of at least 100,000 Da. The void volume fractions from both strains had the sugar composition expected for K30 polysaccharide (Fig. 1A), with a mannose/galactose/glucuronic acid ratio of 1:2:1. Electron microscopy also showed the presence of high-molecular-weight CPS on the cell surface (Fig. 3). Antibody-stabilized capsules of both PR3358 and PR3957 were indistinguishable from that of the parental strain CWG44 with respect to surface association, integrity, and size.

The absence of K_{LPS} in LPS core-defective mutants (see above) and the properties of K_{LPS} (16, 58) argue strongly for substitution of the *E. coli* O9:K30 core with two different polysaccharide surface antigens: the neutral polymannose O9 antigen and the acidic K30 antigen. However, elaboration of high-molecular-weight K30 polysaccharide to form a capsular structure does not require covalent linkage to the LPS core, nor does it require association with K_{LPS}.

K_{LPS} in other *E. coli* strains with group I K antigens. To determine whether K_{LPS} is a general characteristic of *E. coli* strains with group I CPSs, as suggested by Jann and Jann (18), LPS from proteinase K-treated, whole-cell lysates of strains from a variety of group I K serotypes was examined by Tricine–SDS-PAGE (Fig. 4). All of the strains examined were

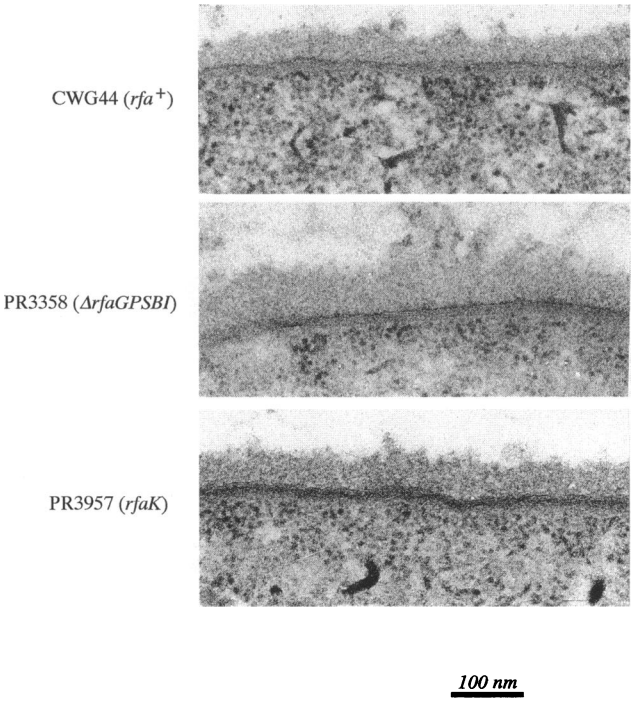

FIG. 3. Effect of LPS core defects on the production of a K30 capsule structure. The micrographs show the cell surface of *E. coli* CWG44 (O^-:K30), PR3358 (*rfaGPSBI*), and PR3957 (*rfaK2*). The K30 capsules were stabilized for electron microscopy by treatment with MAb 4-15A.

smooth (O^+) and produced either O8 or O9 O antigen seen as a characteristic, tightly clustered series of high-molecular-weight bands. In addition, all strains produced a band with the same mobility as the unsubstituted R1 LPS core of CWG28 (O^+K^-). Most, but not all, strains had bands with mobilities between that of the unsubstituted core and that of the high-molecular-weight O antigen. These bands are thought to be K_{LPS}, by analogy with *E. coli* strains O9:K30 (58) and O8:K40 (19), for which the association of K antigenicity with K_{LPS} is documented. These bands are not due to semirough O8 or O9 LPS, because preparations of these LPSs do not contain semirough molecules (19, 21, 58). In the O9 prototype strain (CWG28), no low-molecular-weight O9-substituted LPS molecules are detected with mobilities similar to K_{LPS} (58), and similar results were reported for *E. coli* O8:K40 (19). Finally, the interband spacing of the K_{LPS} molecules in Tricine–SDS-PAGE is proportional to the size of the repeating unit in strains with known K antigen structures.

All strains with group IB (amino sugar-containing) K antigens produced some K_{LPS}, and the degree of polymerization varied from 1 (K45) to >12 (K47). By contrast, only some of the group IA strains (no amino sugars in the K antigen) had K_{LPS}. K_{LPS} was not found in the LPS profiles of serotypes K27, K28, K29, K34, and K37, and only trace amounts were seen in K26. Furthermore, the degree of polymerization was generally less than that seen in strains with group IB K antigens; most group IA strains showed a maximum of a single K_{LPS} band. However, the absence of K_{LPS} in silver-stained, proteinase K-treated whole-cell lysates does not preclude the presence of trace amounts of K antigen attached to the LPS core. LPS profiles of O8:K40 show slightly higher degrees of polymerization when examined by immunoblotting (19).

Although lipid A-core is normally the acceptor for O antigen, it may be an acceptor for other cell surface carbohydrate antigens. These include group I K antigens (described above and in reference 19) and the ECA (28, 31). Although ECA_{LPS} is seen only in *E. coli* rough mutants of strains with core type R1, R4, or K-12 and the levels of core substitution with ECA are low enough to frequently require immunoblotting for detection (ca. 5% [28, 31]), K_{LPS} is seen in strains expressing O antigen, and the level of substitution is often high enough to permit detection by silver staining (Fig. 4). However, for both K antigen (described above and in reference 19) and ECA (31), a large portion of the antigen is expressed in a high-molecular-weight form not associated with LPS. High-molecular-weight ECA is covalently linked to an L-glycerophosphatide which serves as a membrane anchor (32). Similar lipid anchors are seen at the reducing end of *E. coli* group II K antigens and at the reducing ends of the CPSs of *N. meningitidis* (12, 52) and *H. influenzae* (52). In *N. meningitidis* CPS, lipid modification is required for export of nascent group B CPS across the cytoplasmic membrane (7). However, phospholipid substitution of the group II and group II-like extracellular CPSs is nonstoichiometric, and it has been suggested that association of unsubstituted CPS with the cell surface may be mediated by electrostatic interactions (salt bridges) with lipid-substituted CPS (18).

The association of group I CPS with lipid A-core has led to the suggestion that LPS may serve as a membrane anchor (18, 19). As for group II CPSs, group I CPS not covalently linked to lipid A-core could be maintained at the surface by electrostatic interactions (salt bridges) with the lipid-substituted CPS. The phenotypes of the LPS-defective mutants PR3358 and PR3957 and the absence of detectable K_{LPS} with several other group IA CPSs (Fig. 2) indicate that K_{LPS} is not necessary for such associations. However, the data do not preclude a similar interaction between CPS and the inner region of lipid A-core. The means by which high-molecular-weight group I polysaccharides are anchored to the cell surface remain unknown. Electrostatic interactions with other cell surface components are possible because large amounts of K30 CPS can be recovered from wild-type bacteria by saline extraction (17). Alternatively, a lipid substituent analogous to that seen with ECA or the group II CPSs may be present. Trace amounts of fatty acids can be detected in gel filtration-purified high-molecular-weight K30 CPS, but covalent linkage to the K30 antigen is difficult to determine because of the large size of the polymer relative to the putative anchor (53).

It should also be noted that surface carbohydrates (O antigen) normally associated with LPS can be expressed on the cell surface without covalent linkage to the LPS core. Polysaccharides with chemical structures identical to the O antigen of the cell but not attached to the LPS core (O antigen capsule) have been described for *E. coli* O111 (9, 11, 49), O55, and O127 (49) and for *Proteus mirabilis* O6 (1). Surface expression of O antigen in *S. enterica* serovar Typhimurium requires ligation of O antigen to lipid A-core (41), and no O antigen capsules are found in *S. enterica* serovars Typhimurium and Minnesota (49). However, in *E. coli* O111 there appear to be two independent pathways for surface expression of O111 polysaccharide, because pulse-chase experiments indicate there is no precursor-product relationship between O111 LPS O antigen and O111 O antigen capsule (11). Mutations which affect O antigen capsule production but do not impair LPS-associated O antigen have been described (9). Surface expression of K30 antigen also appears to occur via two independent pathways, although K_{LPS} in group I strains of *E. coli* is much smaller than either O antigen or O antigen CPSs.

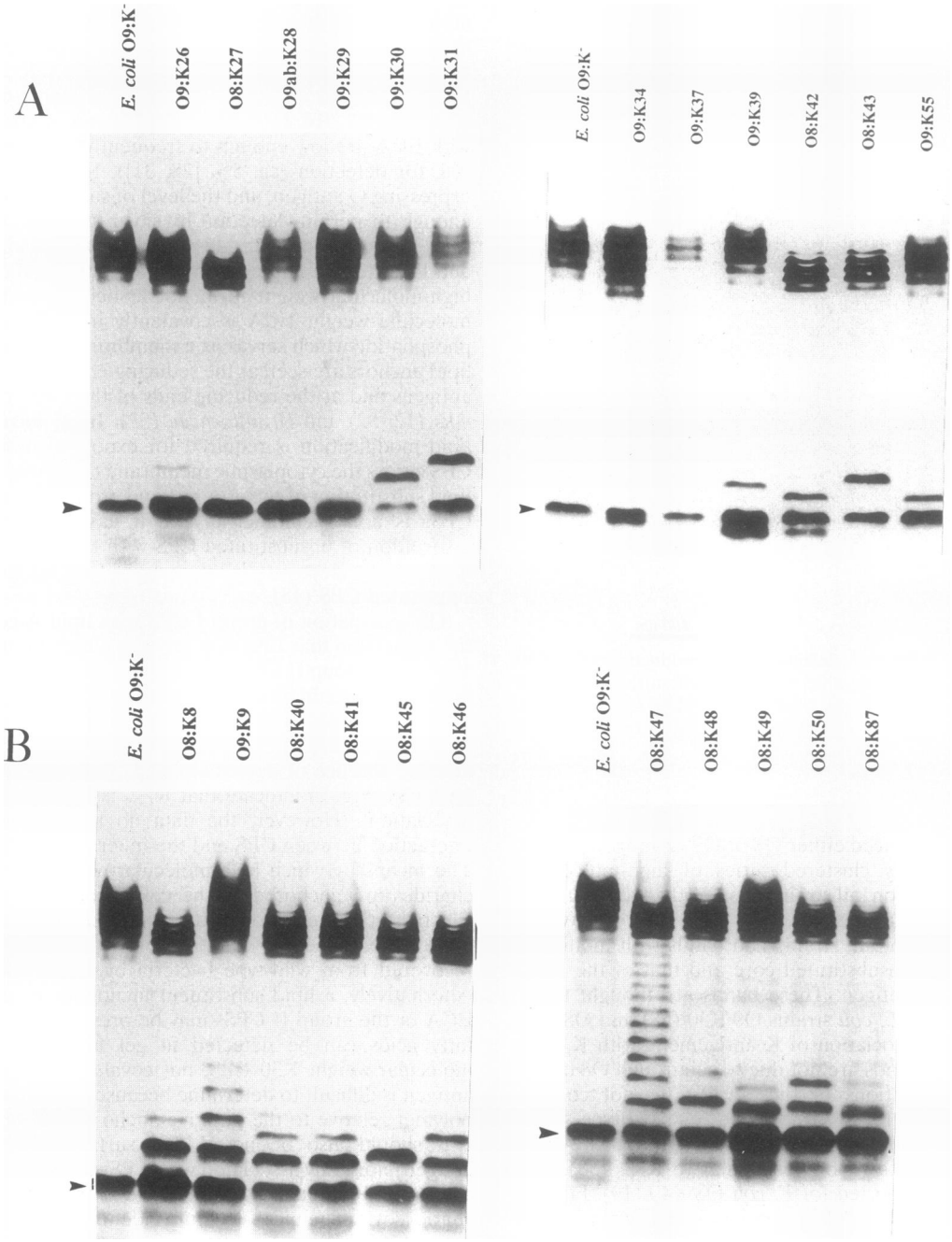

FIG. 4. Tricine–SDS-PAGE of LPS from *E. coli* strains with group I K antigens. LPS was prepared from the strains indicated above the lanes by the SDS-proteinase K whole-cell lysate method (14). The upper panels (A) show LPS from strains with group IA K antigens. LPS from strains with group IB K antigens is shown in the lower panels (B). Arrowheads indicate the positions of molecules containing a complete R1 core in *E. coli* CWG28 (O9:K$^-$). The O8- and O9-substituted LPS molecules migrate slowly as a tight cluster of bands. K_{LPS} bands migrate behind the R1 core band.

In addition to the serotype-specific O and K antigens, several members of the family *Enterobacteriaceae*, including many *E. coli* strains, produce the non-serotype-specific exopolysaccharide colanic acid (the M antigen) (39, 45). Because colanic acid is not serotype specific and because it is not tightly associated with the cell surface via a specific lipid substituent, it has traditionally been omitted from K antigen classification schemes. Several observations suggest that colanic acid should be considered a group IA CPS. First, genetic loci for colanic acid (55) and group IA K antigens (34, 51, 58) both map near the *his* locus (44 min on the *E. coli* K-12 linkage map) and, in the case of K30 CPS (group IA) and colanic acid, these loci may be allelic (27). Second, both colanic acid and K30 are regulated by highly homologous *rcs* regulatory systems (13, 25,

27). Finally, as indicated here, because the presence of significant levels of association with lipid A-core is neither a universal property of group I CPSs nor is essential for surface expression of at least K30 CPS, the nature and degree of cell association may not be good criteria for exclusion.

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