

CROSSREACTIONS OF *ESCHERICHIA COLI* K AND O POLYSACCHARIDES IN ANTIPNEUMOCOCCAL AND ANTI-SALMONELLA SERA

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The study of crossreactivity in relation to chemical structure of the microbial polysaccharides has shown both theoretical and practical utility. In this paper, we make use of recently determined structures of *E. coli* K and O polysaccharides to derive new relationships of structure and specificity, and to update some of the data in earlier work.

Materials and Methods

These have been described in earlier papers (1-3). Pneumococcal (Pn)¹ type-numbers are given in Roman type to avoid confusion with those of *E. coli* and *Klebsiella*.

Results and Discussion

Data obtained are summarized in Table I.

E. coli K Polysaccharides. K2 has 1,4-linked D-galactose (D-gal) and 1,5-linked D-galf (furan form of D-galactose; all sugars are pyranose form unless otherwise stated) in addition to glycerophosphate residues in its repeating unit (4). Precipitation (++±) in anti-Pn XII (see Table I) may be caused by a loose fit of glycerophosphate into antibody sites designed for the D-N-acetylmannosaminic acid (D-manNAcA) of Pn soluble specific capsular polysaccharide (PnS) XII (5, 6). PnS XXIX has two 1,6-linked D-galf residues in its repeating unit (7). Since carbons 5 and 6 of galf are outside the furanose ring, it is possible that the 1,5-D-galf of *E. coli* K2 would fit partially into antibody sites in anti-Pn XXIX designed for 1,6-linked D-galf residues, to give the ++ precipitation found. There was also ++ in anti-Pn XVI, but the structure of PnS XVI is not known.

K4 is omitted from Table I, as it gave only a single + reaction (on a scale of - to +++) in anti-Pn XXII serum.

K5 has the repeating unit $\rightarrow 4$ glcNAc- α -(1 \rightarrow 4)glcA- β -(1 \rightarrow _n) (glcNAc, N-acetylglucosamine; glcA, glucuronic acid) (8). Its faint (+) crossprecipitations were in anti-Pn III, IX, XII, XVI, and XXV.

K7 has the repeating unit $\rightarrow 3$ -D-manNAcA- β -(1 \rightarrow 4)-D-glc- β -(1 \rightarrow _n) (glc, glucose) (9). It reacts with so many equine anti-Pn sera that at least some of the

¹ Abbreviations used in this paper: f, furan form; Pn, pneumococcal; PnS, Pn soluble specific capsular polysaccharide.

reactions might be due to previous or inapparent infections of the horses by strains of *E. coli* containing K7 or a crossreacting antigen such as the enterobacterial common antigen (ECA), which also contains D-manNAcA in its repeating unit (10), and has shown some unexpected crossreactions. Nevertheless, some of the precipitations appear to be correlated with known structural features of Pn polysaccharides. The 1,3-linked D-manNAcA of K7 probably reinforces a cross-reaction in anti-Pn III due to 1,4-linked D-glc, since it would be expected to have some of the serological properties of the D-glcA of PnS III $\rightarrow 3$ - β -D-GlcA-(1 \rightarrow 4)- β -D-glc(1 \rightarrow \bar{n}) (sugars are capitalized only when known to be immunodominant). For examples of the partial serological equivalence of glc, glcNAc, and manNAcA, cf. previously published data (11-13). K7 precipitates 242 μ g/ml of antibody nitrogen from anti-Pn III 792C, far more than the +++ in anti-Pn VIII 1008 of higher antibody content. The repeating unit of PnS VIII is half cellobiouronic acid (14), and the remainder is D-glc \rightarrow D-gal (galactose), but all linkages are 1,4, instead of the 1,3-linked D-glcA of PnS III $\rightarrow 3$ -D-GlcA- β -(1 \rightarrow 4)-D-glc- β -(1 \rightarrow \bar{n}) (15), so that the greater reactivity in anti-Pn III is in accord with the three structures.

PnS II, XIV, XV, XIX, XXII, and XXIII contain 1,4-linked D-glc in their repeating units: the crossprecipitations in antisera to these types appear due (16) to multiples of these residues, reinforced in the large reaction (99 μ g/ml of antibody nitrogen) in anti-Pn XIX by 1,4-linked D-manNAc (D-N-acetylmannosamine), a determinant of PnS XIX (17). Precipitation in anti-Pn I, VI, IX, and X may be caused by inapparent crossreacting *E. coli* infections of the immunized horses, as indicated above.

K8 is made up of gal, glcA, galN (galactosamine), and glcN (glucosamine) (18). The reaction was only ++ in anti-Pn XXII, + in anti-*Salmonella typhi* and anti-*S. paratyphi* A, and ++ in anti-*S. paratyphi* B.

K9 (18, 19) contains gal, N-acetylneuraminic acid, and N-acetylgalactosamine (galNAc). It gave a + reaction in anti-Pn I, III, VIII, IX, XV, and XXV and \pm in anti-Pn VI, VII, and X. In the last three, common linkages of D-gal and/or galNAc in the relevant polysaccharides probably give rise to antibodies producing the slight crossprecipitations.

K12 (20) showed a + reaction in anti-Pn V and XXVII.

K14 (21) and 17 were uniformly - to \pm and, with K12, are not included in Table I. Immunodominance of partially O-acetylated 2-keto-3-deoxymannosoc-tonic acid (KDO), which does not occur in PnS, may explain the lack of reactivity of K12 and 14 in anti-Pn sera.

K25 is made up of glcA, galNAc, and fucose (fuc) (18, 19). It crossreacts weakly in anti-Pn V and MXXV. Possibly, 1,2-linked D-glcA will be found in K25, as it occurs in PnS V (22); the structure of PnS XXV is not known.

K26 contains glcA, gal, and rhamnose (rham) (18, 19). Strong crossprecipitation in anti-Pn II and XXIII indicates that its repeating units contain nonreducing lateral end groups of D-glcA, as in PnS II (23), and of L-rham, as in PnS XXIII (24-26). With these structural similarities to K85 (see below), there should be reciprocal crossreactivity between K26 and K85 and antisera to these, but this does not seem to have been looked for. The reaction in anti-Pn VI is

TAB
Crossreactions of *E. coli* Polysaccharides in

E. coli antigens	Antipneumo												
	I 1024	II 4000	III 600	IV 2390	V 4060	VI 724	VII 893	VIII 1288	IX 1655	X 864	XI 792	XII 1240	XIV 1010
K2	-	±	±	-	±	±	±	-	±	-	-	+++	±
K7	+++	+±	242	+	-	++	+	+++	+++	+±	±	±	++
9	(+)*	(-)	(+)	(±)	(-)	(±)	(±)	(+)	+	(±)	(-)	(-)	(-)
25	-	-	-	-	+++	-	-	-	-	-	-	-	±
26	-	300	-	-	-	+++	-	-	-	-	-	-	±
27	+±	-	+±	+	+	-	-	-	±	++	-	+	±
28	-	++	+	±	+	±	+++	+	±	++	-	±	±
30	-	505 [‡]	-	-	145 [‡]	-	-	-	-	-	(-)	(-)	(±)
31	(-)	(-)	(+)	(±)	(±)	(+)	(+)	(-)	(-)	(+)	(-)	(-)	-
42	8	±	±	-	-	±	±	-	-	±	±	±	±
54	-	±	++	-	-	+++	-	-	-	-	-	-	-
57	+++	-	-	-	-	-	-	-	-	-	(-)	(-)	(-)
85	±	181 [‡]	-	-	459 [‡]	(+)	-	-	-	28 [‡]	+±	-10±	-10±
87	(-)	-	(-)	(-)	-	(-)	(±)	18 [‡]	(+)	(±)	(-)	(-)	+
(Ø147K) 100	++	-	±	++	±	+++	+	+	++	++	-	-	+
O8 [‡]	+	-	±	±	±	+	+	±	++	++	±	±	++
O8LPS	(++)	(++)	(+)	(±)	(±)	(±)	(±)	(+)	(+)	(+)	(+)	(±)	(++)
O8PS	+++	+	±	±	-	-	-	-	±	+++	±	±	-
O9	(±)	+±	(±)	(-)	(±)	(±)	(±)	(+++)	(+)	(+++)	(+++)	(++)	(-)
O13 [‡]	(±)	(+++)	(+++)	(++)	(+)	(++)	(++)	(+)	(+)	(±)	(++)	(+++)	(+++)

* Readings in parentheses: tests made in two or more rows of antisera that were - to ++ or +++ in other polysaccharides.

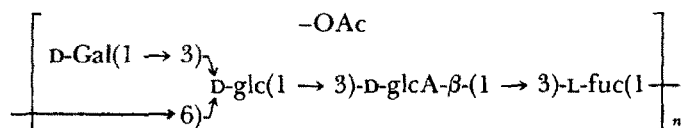
[‡] *E. coli* K26 precipitated Ranti-K47 *Klebsiella*. K1 47, like PnS XXIII, has lateral nonreducing end groups of L-rham.

[‡] From Heidelberg et al. (37).

[‡] +++ in RXVII.

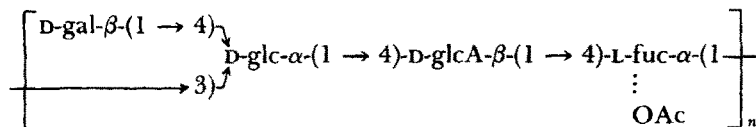
probably due to 1,3-L-rham and/or to 1,2-D-gal. The explanations of these crossreactivities in Ørskov et al. (18) are thus extended and modified.

K27 has the probable (27) structure:



Weak precipitation in anti-Pn I is difficult to explain. Failure to react in anti-Pn XIV may indicate that -OAc is on the gal. PnS X (28) and XX (29) have lateral nonreducing end groups of D-galf. Crossreactions in anti-Pn X and XX might indicate that the lateral gal of K27 is also galf, although gal (pyran) and galf are not necessarily noncrossreactive.

K28 has (30) the structure:



L-fuc is acetylated at positions 2 or 3 in 70% of the repeating units. It is difficult to see why K28 should give even ++ in anti-Pn II or why, if reactivity in anti-Pn VII is due to the lateral D-gal, there is no definite precipitation in anti-Pn

LE I

Antipneumococcal and Anti-Salmonella Sera

coccal sera											Anti-Salmonella sera		
XV 770	XVI 872	XVIII 2200	XIX 2250	XX 355	XXII 878	XXIII 420	MXXV 620	XXVII 277	XXVIII 785	XXIX 389	typhi	paratyphi A	paratyphi B
-	++	+	-	+	±	±	±	±	±	++			
+++	-	-	99	±	11	+++	++ [§]	-	±	+			
+	(-)	(-)	R(-)	(±)		(-)	(+)	(-)	(±)	(±)			
-	±	-	-	±		-	+±	-	+	-			
-	-	-	-	-	+±	197 [‡]	-	-	-	-			
-	+±	+	-	+±		-	+±	+	±	-			
-	-	-	±	±	±	±	+	±	-	+	-	+	-
(-)	(-) [¶]	(±)	(-)	(-)		-	±	-	-	-	-	-	-
-	-	-	-	-	(±)	(+++±)	- or ±	- or ±	- or ±	- or ±	- or ±	- or ±	- or ±
±	±	±	-	-	-	-	152 ^{**}	-	-	-	±	-	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-
(-)	(-)	(-)	(-)	(-)		(±)	(±)	(±)	(-)	(+)			
- to ±	- to ±	- to ±	- to ±	- to ±	- to ±	20 [§]	±	±	±	-			
- to ±	- to ±	- to ±	- to ±	- to ±	- to ±	-	+	-	-	-			
-	-	-	-	+++	+++	-	-	-	-	-	+++	-	±
±	±	-	+±	+±	++	+	±	+	+	+	++	-	+++
(+)	(-)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
±	-	+	++	+++	+++	-	-	++	-	+	+++	++	+++
(-)	(-)	(++)	(±)	106		-	±	-	-	-			
(++)	(+±)	(++)	(++)	(+±)		+++	±	-	-	-		++	

[†] Tests with equine anti-Pn XXV 513C (New York City Department of Health).

^{**} From Heidelberg et al. (37) table I, footnote d, (278 - 126 = 152).

[‡] 27 µg after treatment with alkali.

[§] From G. Springer, Northwestern University School of Medicine, Evanston, IL.

[¶] From A. Zweibaum, Hôpital Broussais, Paris.

XII or XIV; the +± reaction in anti-Pn X is probably for the reason given for K27.

K29 contains 1,3-linked glucosyl residues in its repeating unit (31). These are probably responsible for the ++ in anti-Pn VI. There should also be a heavy crossreaction with *Klebsiella* (Kl) K31, which has the same pyruvyl (Py) 4,6-glc-(1 → 2)-D-man sidechain (32), and perhaps also with Kl 36 (33) and Kl 64 (34), which also have 4,6-Py-glc nonreducing lateral end groups.

Crossreactivity of K30 (35) and K85 (36) in anti-Pn II and V, and of K85 in anti-Pn XXIII was expected, found, measured, and discussed previously (37). It may now be added that, since K85 precipitates more antibody from anti-Pn V (PnS V has 1,2-linked D-glcA [22]) than from anti-Pn II, this would favor assignment of the 1,2-linkage to the nonterminal glcA of K85, rather than the 1,4-linked alternative also proposed (18, 37). Recent work (28) has shown that pure PnS X contains no glcNAc, so that the crossreaction of K85 in anti-Pn X can not be attributed to this sugar, as was done previously (18, 37). Alternatively, the glcA residues of K85 might fit partially into combining sites of anti-Pn X designed for reception of the ribitolphosphate residues of PnS X.

Recently (38), *Klebsiella* K63 was found to contain the same sugars in the same linkages as *E. coli* K42 (39). Quantitative analyses (1), however, show that Kl 63 precipitates 148 µg/ml of antibody nitrogen from anti-Pn I 1057C whereas *E. coli* K42 gives only 8 µg/ml (37). Although the primary structures of the two polysaccharides are the same, Kl 63 is said to contain <0.2 residues of -OAc per

repeating unit, while *E. coli* K42 has 0.5. Assuming that K42 has not been partially depolymerized or degraded, one might explain the analytical data as follows. If the -OAc of K42 were on galA, and antibodies in anti-Pn I were partly directed against unacetylated galA, the discrepancy would be accounted for. Alternatively, the three-dimensional form of the more highly acetylated K42 might differ from that of Kl K63. Strictly, then, *E. coli* K42 and Kl 63 are not identical.

K31 (described by K. Jann and B. Jann, unpublished results), with its only heavy crossreaction in anti-Pn XXIII, provides another instance of a 1,2-linked sugar acting serologically much like a nonreducing lateral end group. In the repeating unit of K31, it is the 1,2-linked L-rham that reacts like the L-rham in K26 (above).

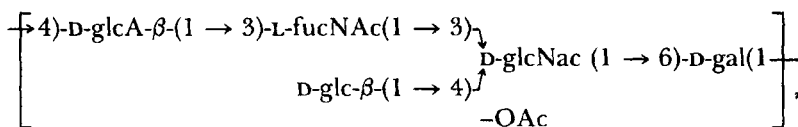
K51 is omitted from Table I because the only significant reaction, (++) in anti-Pn XVIII, is not interpretable with the information at hand.

K52 is also omitted, as all tests showed little (+) or no (-) crossreactivity.

K54 has a unique repeating unit: $\rightarrow 3$ -D-glcA-L-threonylamide- β -(1 \rightarrow 3)-L-rham- α -(1 $\overline{\rightarrow}$ (40) (occasional units have serine instead of threonine). From its crossprecipitation in anti-Pn III and not in anti-Pn VIII, predicted earlier that the glcA would be D-, and 1,3-linked, as in PnS III, and not 1,4-linked as in PnS VIII. Amidation of glcA seems not to have affected its specificity. 1,3-linked L-rham explains the crossreactivity in anti-Pn VI (41).

K57 contains gal, ribose, galA, and galNAc (18). Its only significant (+++) crossprecipitation, in anti-Pn I, appears due to D-galA in a 1,3- or 1,4-linkage, as in PnS I (42).

K87 has (43) the repeating unit,



The strongest crossprecipitations (+±) were in anti-Pn VII and 18 μg/ml antibody nitrogen in anti-Pn VIII, the latter value increasing to 27 μg after treatment of K87 with alkali. Precipitation of anti-Pn VIII is undoubtedly caused by the existence of D-glcA in K87 and PnS VIII (14) in 1,4-linkage.

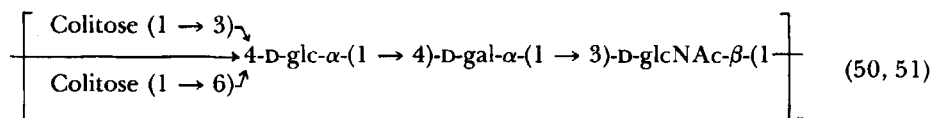
K100 gave crossreactions not exceeding ++± in anti-Pn I, IV, VI, IX, X, XX, XXII, and anti-*S. typhi*. Since K100 is said (44) to be identical to the polysaccharide of *H. influenzae* b, $\rightarrow 3$ -D-ribosyl- β -(1 \rightarrow 1)ribitol-5-phosphate $\overline{\rightarrow}$ (45), it is possible that the precipitation in anti-Pn VI and X might be due to the known occurrence of ribitolphosphate as part of the repeating units of PnS VI and X. Or, as in other instances recorded herein, the horses used for production of antisera might have had inapparent infections with a K100-containing or cross-reacting microorganism.

O Polysaccharides and Lipopolysaccharides. Three different preparations of O8 were tested, as well as one of O9. Since 60% of the repeating unit of O9 consists of the repeating unit of O8 (46, 47) it is surprising that there is no crossreactivity between these types (48). However, one of the 1,2-mannosyl residues on O8 is β -linked (Jann, Jann, and Himmelspach, unpublished observations), which might

bend the molecule into a shape different from that of O9, in which all linkages are α . The reaction of O9 in anti-Pn XII may be caused by its multiples of α -1,2 mannobiosyl residues. Since mannose is partially equivalent serologically to glucose (12), O9 could fit loosely into antibody sites designed to bind the kojibiosyl residues of PnS XII. The number of relatively weak crossprecipitations in anti-Pn sera may be due, as in other instances, to inapparent infection of the immunized horses with O8, O9, or to crossreacting strains.

Cells of *E. coli* O13 yield antisera in rabbits that precipitate heavily with glycogen (49). A glc-containing O13 polysaccharide also sent by Dr. A. Zweibaum (Hôpital Broussais, Paris, France) reacts to various extents with anti-Pn I-XX, so that it is questionable whether all of the precipitates involve type-specific antibodies.

O111 has the structure.



This polysaccharide, from Dr. O. Westphal (University of Freiburg, Federal Republic of Germany) contains colitose, glc, gal, and glcNAc. It was tested in all listed antisera, giving only very weak (+) to negative (-) reactions, and is therefore omitted from Table I. After degradation with 1% acetic acid, at 100°C for 90 min, which removes both colitose residues from the glc, it precipitated 60 $\mu\text{g/ml}$ of antibody nitrogen from anti-Pn VIII 1008, confirming the presence of 1,4-linked D-glc in O111.

Summary

Crossreactions of 24 K polysaccharides and 4 O polysaccharides of *E. coli* in antisera to 27 pneumococcal types, 3 anti-*Salmonella* sera, and anti-*Klebsiella* K1 serum are discussed in relation to structural features of the polysaccharides insofar as these are known. Predictions based on the crossprecipitations are also ventured for several instances in which structures are as yet undetermined.

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