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Relations Between Structures of Three K Polysaccharides of *Escherichia coli* and Cross-Reactivity in Antipneumococcal Sera

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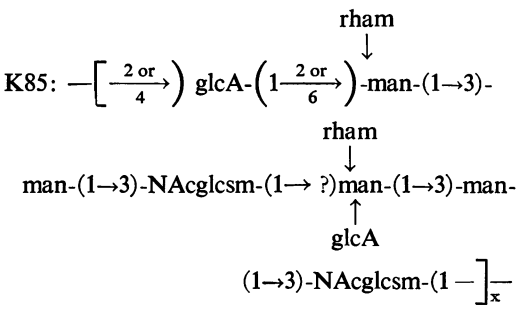
Evidence has been presented for the following structures of the K polysaccharides of *Escherichia coli* O9:K30:H12 (D. Hungerer et al., European J. Biochem. **2**:115, 1967), O8:K42(A):H$^-$ (K. Jann et al., Biochem. Z. **342**:1, 1965, and O141:K85(B):H4 (K. Jann et al., Biochem. Z. **346**:368, 1966):

K30: —[→ 3)-man-(1 → 2)-β-D-glcA-
(1 → 3)-D-gal-(1—]$_x$—

K42: —[→ 3)-D-gal-(1 → 3)-D-galA-
(1 → 2)-fuc(*f*)-(1—]$_x$—



We have examined a number of cross-reactions of these substances, in most instances quantitatively (M. Heidelberger and P. A. Rebers, J. Am. Chem. Soc., **80**:116, 1958; M. Heidelberger and J. M. Tyler, J. Exptl. Med. **120**:711, 1964) (Table 1). In general, the analyses supported the assigned structures and furnished additional information as to the immunological determinant groupings involved.

Reactions of K30. In the Pn VI system, 1,2-linked D-galactose has some of the immunological properties of nonreducing end groups of this sugar (M. Heidelberger and P. A. Rebers, J. Bacteriol. **80**:145, 1960). If this analogy is applicable, it is not surprising that K30 reacts in anti-Pn II and V much as do polysaccharides with nonreducing end groups of D-glucuronic acid (M. Heidelberger, J. Exptl. Med. **111**:33, 1960; Arch. Biochem. Biophys. Suppl. **1**:169, 1962). The D-glucuronic acid of S V (pneumococcal capsular polysaccharides are designated S, with the appropriate type number) is said to be linked 1,2- (S. A. Barker et al., Carbohydrate Res. **2**:224, 1966), as in K30, so that one might expect K30 to react in the two antisera as does K85 (*see below*). The action of alkali on K30 was originally interpreted as removal of OAc and splitting of interchain linkages, effects which may be responsible for the reduction in the amount of antibody precipitated from anti-Pn V after treatment of K30 with alkali. The much stronger reaction in anti-Pn II 513 remained unaffected. Precipitation in both antisera was abolished by partial or complete oxidation of K30 with periodate, whereas a very weak positive reaction of K30 in anti-Pn XIV, ascribed to the 1,3-linked D-galactose in the repeating unit, survived at least partial oxidation. K30, alkali-treated K30, and partly oxidized K30 failed to precipitate horse anti-*Salmonella typhi* serum 834 (for which we are indebted to A. M. Staub); the fully oxidized and reduced product gave a weak reaction, as would be expected from its content of multiple residues of 1,3-linked mannose (M. Heidelberger and F. Cordoba, J. Exptl. Med. **104**:375, 1956; M. Heidelberger and A. Jeanes, J. Bacteriol. **86**:881, 1963).

Reactions of K42. The weak cross-reaction in anti-Pn I appears to be due to 1,3-linked D-galacturonic acid, which also occurs in Pn S I (R. C. F. Guy et al., J. Biol. Chem. **242**:5106, 1967). The only strong cross-reaction in the whole series of antisera tested was in anti-Pn XXV. S XXV had $[\alpha]_D$ + 128° and contained uronic acid, amino sugar, and acetyl (R. Brown, J. Immunol. **37**:445, 1939). A chromatogram run in this laboratory by A. Das showed spots with the mobilities of galactose, galacturonic acid, glucos

TABLE 1. *Cross-reactions of K30, K42, and K85 polysaccharides in antipneumococcal horse sera at 0 C*

Anti-Pn: Polysaccharide	Antibody N precipitated (μg, calculated to 1.0 ml of antiserum)						
	I 884C[a] (970)[b]	I 1057C[a] (1,024)	II 513 (3,600)	V 555 (4,060)	X 627C[a] (864)	XXIII 912 (275)	XXV 513C[a] (186)
K30							
100 μg				135			
200 μg				145			
250 μg			395				
500 μg			495, 515				
1,000 μg			440				
Alkali-treated K30							
100 μg				69[c]			
200 μg				96[c]			
400 μg				86[c]			
500 μg			485				
1,000 μg			455				
K42							
25 μg	8	8					
50 μg	3	3					
100 μg							265[d]
200 μg							290, 265[d]
K85[e]							
50 μg					23	20	
100 μg			121, 167		28	18	
200 μg			160				
300 μg			181	456			
500 μg				465,[f] 453			
Partially oxidized K85							
50 μg					70[g]		
100 μg			75		68		
200 μg			82				
250 μg				76			
500 μg				89			
Fully oxidized K85							
50 μg					70[h]		
100 μg					68		

[a] Absorbed with pneumococcal C-substance. The other antisera contained negligible amounts of anti-C.

[b] Numbers in parentheses represent maximal percipitable type-specific antibody N.

[c] Supernatant fluids + partly oxidized K85 gave 32 μg of nitrogen.

[d] Supernatant fluids + S XXV gave 68 μg of nitrogen. Anti-Pn XXV, after precipitation at the maximum with S XXV, still gave 126 μg of nitrogen with K42.

[e] Alkali-treated K85 gave similar values in anti-Pn II and V, the only sera tested.

[f] Supernatant fluids gave no precipitate with K30.

[g] Reaction inhibited only 12% by *N*-acetyl-D-glucosamine at the 90 μM level; 0.40 ml of antiserum; total volume, 0.83 ml.

[h] Inhibited 9% by NAc-D-glucosamine at the 45 μM level; 0.50 ml of antiserum; total volume, 0.73 ml.

amine, and galactosamine. Strangely enough, K42 precipitated more nitrogen from anti-Pn XXV than did three different preparations of S XXV. This was also true of a birch sap polysaccharide (B. Urbas, G. A. Adams, and C. T. Bishop, Can. J. Chem. **42**:2093, 1964). At least one-half of the nitrogen precipitated by K42 was type-specific antibody-nitrogen precipitable by S XXV, as shown in footnote *d* of Table 1. Perhaps S XXV is more easily depolymerized during its isolation than is usual with the pneumococcal polysaccharides. No infection was noted during the immunization of horse 513 (P. S. May, *personal communication*).

Reactions of K85. As expected from the assigned structure, the strongest cross-reactions of K85 were in anti-Pn II and V. K85, however, is unique in being the only substance found thus far, aside from S V itself, which reacts more strongly in anti-Pn V than in anti-Pn II. Since D-glucuronic acid is the only sugar common to K85 and S V, it seems that the residues of this sugar in K85

would more nearly approximate those of S V spatially than those of S II. Further study of the fine structures of all three polysaccharides is desirable. The diminution of both cross-reactivities on partial oxidation and their complete disappearance upon oxidative destruction of practically all of the D-glucuronic acid agree with the proposed structure of K85.

The reverse behavior of cross-precipitation in anti-Pn X is consistent with the assignation of 1,3-linkages to *N*-acetylglucosamine in K85, and with Z. A. Shabarova, J. G. Buchanan, and J. Baddiley's finding (Biochim. Biophys. Acta **57:** 146, 1962) that R. Brown's S X is made up of galactose, glucosamine, galactosamine, and ribitol phosphate. Oxidation of K85 for 5 hr, which destroyed all of the rhamnose and 60% of the glucuronic acid, was sufficient to produce the maximal increase in this cross-reaction. Therefore, it seems that (i) *N*-acetylglucosamine in both K85 and S X occurs in the same enantiomorphic form, and (ii) at least a portion of the *N*-acetylglucosamine in S X is linked 1,3-, as in K85.

The precipitation of anti-Pn XXIII by K85 is undoubtedly due to nonreducing end groups of L-rhamnose in the repeating units of K85 and S XXIII (J. K. N. Jones and M. B. Perry, *unpublished data*; M. Heidelberger, J. M. Davie, and R. M. Krause, J. Immunol. **99:**794, 1967). Partially oxidized and reduced K85 and fully oxidized and reduced K85 failed to precipitate in anti-Pn XXIII; in both products, the rhamnose residues were destroyed. Since these residues in S XXIII were identified as L-rhamnose, this also establishes the L configuration for at least a part of the rhamnose in K85.

Footnote *f* of Table 1 shows that a portion of the same fraction of anti-Pn V which is reactive with K85 is precipitated by K30; footnote *c* shows that the residual glucuronic acid of partly oxidized K85 is sufficient to cause precipitation in supernatant fluids absorbed with alkali-treated K30.

We believe that the simple quantitative immunochemical analyses cited in this paper have extended the present knowledge of antigenic determinants in both of the large classes of microorganisms involved.

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