CROSS-REACTIONS OF POLYSACCHARIDES OF *Lipomyces* IN ANTIPNEUMOCOCCAL AND OTHER ANTISERA*†

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ABSTRACT

Cross-reactions of the extracellular polysaccharides of *Lipomyces lipoferus* and *Lipomyces starkeyi* are described, in some instances quantitatively Non-reducing end-groups of D-glucuronic acid appear to account for most of the explicable reactions of the polysaccharide of *L lipoferus*, whereas nonreducing end-groups of D-galactose account for many of those of *L starkeyi* Because of its strong cross-reaction in antipneumococcal type II serum, the polysaccharide of *L starkeyi* is also presumed to have a portion of its D-glucuronic acid in the form of nonreducing end-groups

NTRODUCTION

Two species of *Lipomyces* are generally recognized *L lipoferus* and *L starkeyi*, although unanimity is lacking as to their differences Slodki and associates have reported that an extracellular polysaccharide produced by *L lipoferus* contains D-mannose, D-glucuronic acid, and *O*-acetyl groups[1] and that a similar polysaccharide of *L starkeyi* is made up of the same components plus D-galactose and not more than 0 5% of D-glucose[2] A different strain of *L starkeyi*, however, extruded a starch-like polymer and a galactomannan[3]

As the order and most of the linkages of the constituent sugars were uncertain, except that *L starkeyi* yielded a di(glucosyluronic acid)mannose and both species an aldobiouronic acid, 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose[1,2], and because studies of the cross-reactivities of polysaccharides had often been helpful in solving problems of structure (for reviews, *cf* Ref 4, 5), the present work was undertaken

*Dedicated to Professor Jean-Émile Courtois, in honor of his 65th birthday

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EXPERIMENTAL

Materials. — The organisms were grown and the polysaccharides isolated as described in previous papers[1-6] Some of the preparations were deproteinized according to Sevag[7] The composition of the polysaccharides used is given in Table I Neither 2-keto acids (as acetals) nor hexosamines could be detected in hydrolyzates of *L starkeyi* and *L lipoferus* polysaccharides Periodate (Smith) degradation of *L starkeyi* Y-1388 polysaccharide indicated the presence of D-galactose and D-mannose units resistant to oxidation

TABLE I

PROPERTIES AND COMPOSITION OF *Lipomyces* POLYSACCHARIDES[a]

Properties and composition	Lipomyces		
	lipoferus *NRRL Y-1351*	starkeyi *NRRL Y-1388*	starkeyi *NRRL Y-2543*
$[\alpha]_D^{25}$ (degrees)[b]	+42	0	−4
O-Acetyl	5 7	5 7	4 0
K D-Glucuronate	27 0	31 2	33 0
Total hexose	51 5	52 8	48 9
D-Galactose[c]		28 7	13 3
D-Glucose[d]	3 3	0 4	0 5

[a]Report as anhydro sugar [b]Optical rotations from Ref 1 [c]D-Galactose was also not detected in *L lipoferus* NRRL Y-2542 and Y-6333 polysaccharides [d]Due to the slight reactivity of D-mannose in the glucose oxidase assay, the actual levels of D-glucose in *L starkeyi* polymers are possibly one-half those given

Deacetylated *L starkeyi* NRRL Y-1388 polysaccharide was prepared from a 0 1% solution with 0 02M sodium hydroxide for 3 h at 25° The product was precipitated with 2 vol of methanol, redissolved in water, dialyzed, and lyophilized The recovered material gave an amount of ferric hydroxamate chromogen equivalent to 5% of the apparent *O*-acetyl groups in the starting material (relative to 1 acetyl group) Since longer treatment with alkali did not alter the yield of residual chromogen, it likely does not indicate *O*-acetyl groups

Methods — The sources of antisera and the methods for the qualitative and quantitative measurement of cross-reactivity have been given in previous papers[8,9]

RESULTS AND DISCUSSION

The polysaccharides of *L lipoferus* (Ll) and *L starkeyi* (Ls) gave cross-precipitation at 0° in many antipneumococcal (anti-Pn) sera, in several anti-*Salmonella* sera, and in an antiserum to *Mycoplasma mycoides* On a scale of − to ++++ Ll reacted ++ or more with anti-Pn II, IV, VI, VIII, IX, X, XIV, XV, XVIII, XIX, XX, XXII, XXIII, XXVIII, anti-*S paratyphi* A and B, Ls with anti-Pn II, VI-XI,

TABLE II

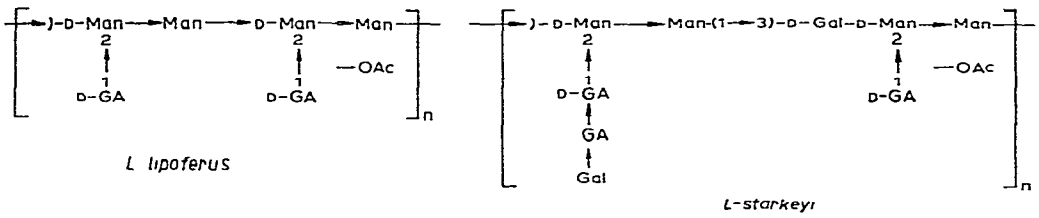
CROSS REACTIVITY OF *Lipomyces* POLYSACCHARIDES IN ANTIPNEUMOCOCCAL SERA[a]

Polysaccharide	*Antipneumococcal sera*[b]											
	II513	*VI681C*	*VII937C*	*VIII1008*	*IX623C*	*RIX91$_2$*	*X627C*	*XIV635C*	*XV628*	*RXVIII163*	*XVIII495C*	*XXII566*
Homologous polysaccharide	3600	724	880	1288	1655	5340	864	1010	770	6860	2200	870
L. lipoferus NRRL Y-1351	855[c]	22[d]	10	18	214	207[e]	50	44[f]	215[g]		173	37
L. starkeyi NRRL Y-1388	624[h]	149[i]	102[j]	44	9[k]		129[l]		443	144	202	
L. starkeyi 1388, rel OAc 1								80			122	
L. starkeyi 1388, rel OAc 05			105					47			0	
L. starkeyi NRRL Y-2543	860						59[m]				12	

[a]Maximal precipitation at 0°, μg of antibody N calculated to 1.0 ml of antiserum [b]Rabbit sera have the designation R, all others are from the horse, C, absorbed with pneumococcal group specific C-polysaccharide [c]Supernatants + S II gave 2800 μg N [d]Supernatants + Dextran 1355 B4 gave 33 μg N, as in intact serum [e]Supernatants from which about 150 μg N had been precipitated by LI gave 68 μg N with isolichenan, intact serum gave 120 μg[10] [f]Supernatants + guaran gave 71 μg N, supernatants after successive precipitation with LI and Ls gave 43 μg N, intact serum gave 81 μg N with the preparation of guaran used [g]Supernatants + Ls 1388 gave 161 μg N, those from anti-Pn XV + a level of *Aerobacter aerogenes* 418 (K2) giving 121 μg N precipitated an additional 207 μg with LI, as much as from intact serum [h]Supernatants, from which Ls had precipitated 500 μg N, + degraded gum arabic gave 234 μg N, intact serum gave 626 (Ref. 11) [i]Supernatants + guaran[13] gave 30 μg N, intact serum gave 106 μg[12] Supernatants from the precipitation of 150 μg N by *Sporobolomyces* acetylphosphogalactan[14] gave only 43 μg N with Ls [j]Supernatants from the precipitation of 150 μg N by streptococcal group F polysaccharide 74, which may have a β D-galactosyl residue as a principal determinant[9], gave only 58 μg N with Ls [k]Supernatants + 100 μg LI precipitated 118 μg N instead of 170 as in intact serum, the supernatants from this gave 500 μg N with isolichenan, intact serum gave 600 (Ref. 10) [l]Supernatants + oxidized-reduced *E. coli* K85 polysaccharide[15] gave 26 μg N, intact serum gave 70 (Ref. 16) [m]Precipitated at the level giving 129 μg N with Y-1388

XIV-XX, XXII, XXIII, anti-*S typhi, paratyphi* A, and *Mycopl. mycoides* Many of these reactions were studied quantitatively and the results are summarized in Table II

Although it would be helpful to have an exact idea of the arrangement of the sugars of which each of the polysaccharides is composed, the available data are too fragmentary for any but tentative structural proposals to serve as models on which the patterns indicated by the cross-reactions could be tested for their fit



Precipitation in anti-Pn II — Heavy cross-reactions of carbohydrates in anti-Pn II have been shown to be due to the presence of multiple non-reducing end-groups of D-glucuronic acid or its 4-*O*-methyl derivative or suitably linked D-glucose[11 17–19] Usually, relatively large amounts of plant or bacterial gums or of glucosans such as glycogen are required for maximal precipitation[11 18] Since Ll and Ls contain minimal amounts of D-glucose (4 and 0 4%, respectively) the multiple residues of D-glucuronic acid are undoubtedly responsible There is also a possibility that positions C-3 to C-6 of the D-mannose residues would fit into the binding sites on the antibody for D-glucose or D-glucuronic acid Mainly because of the strong precipitation in anti-Pn II, a structure having nonreducing end-groups of D-glucuronic acid, in addition to the internal glucuronic acid residues indicated by isolation of di-(glucosyluronic acid)mannose, is attributed to Ls Polysaccharide S II* may have internal residues of glucuronic acid[20–22] so that these may be in part responsible for the effects noted The end-groups in S II are linked α-D-(1→6) to D-glucose[23], any internal residues[20] of glucuronic acid (1→4)

Precipitation in anti-Pn VI — The small percentage (*ca* 4%) of D-glucosyl residues in Ll is not responsible for the slight precipitation in this antiserum (footnote d, Table II) Since Pn S VI contains (1→3)-linked D-glucose residues and neither mannose nor glucuronic acid residues[24], there appears to be no reason why Ll should react

On the other hand, nonreducing end-groups of D-galactose are ascribed to Ls, and polysaccharides with multiples of these have been shown to precipitate anti-Pn VI[12] even though the type-specific antigenic determinant of Pn VI, S VI, is characterized by repeating units containing D-galactose 2-phosphate residues Guar gum and the polysaccharide of *Sporobolomyces* (footnote i) have non-reducing end-groups of D-galactose and D-galactose residues linked at C-1 by a phosphate diester group, respectively[13 14] and precipitate much of the same fraction of anti-Pn VI

*Pneumococcal type-specific polysaccharides are designated S with the appropriate type numeral

Reactivity in anti-Pn VII and anti-Pn VIII — The nonreducing end-groups of D-galactose residues are undoubtedly responsible for the tenfold greater precipitation in anti-Pn VII by Ls than by Ll (see also footnote j) There is evidence that Pn S VII contains β-linked end-groups of D-galactose[25].

Pn S VIII is a linear polymer[26] of D-galactose, D-glucose, and D-glucuronic acid, all (1→4) linked Possibly Ls contains residues of (1→4)-linked β-D-glucopyranosyluronic acid, or (1→4)-linked D-galactosyl, or both as in Pn S VIII

Precipitation in anti-Pn IX — The structure of Pn S IX has not been completely elucidated[27], but the component sugars are D-glucose, D-glucuronic acid, *N*-acetyl-D-glucosamine, *N*-acetylmannosamine, and possibly *N*-acetylgalactosamine[28] The residues of acid appear to be internal and linked in part α-D-(1→3) to D-glucose. There is no obvious reason why Ll should precipitate so heavily in both equine and rabbit anti-Pn IX and Ls negligibly A portion of the reactive antibody is the same as that precipitated by isolichenan (footnotes g, h), a glucan with α-D-(1→3) and (1→4) linkages at least partly in pairs[29 30] Possibly the small amount of D-glucose in Ll is significant in this instance, or again, the rear portions of D-mannose residues may be involved

Reactivity in anti-Pn X — Pn S X is made up of galactose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and ribitol phosphate[31] The considerable cross-reaction of Ll is therefore unexplained, that of Ls would appear to be due to residues of D-galactose similarly linked to those in S X However, the reaction described in footnote l indicates that Ls removed a fraction of anti-Pn X reactive with residues of *N*-acetylglucosamine, a sugar not found in Ls Again, one could fall back on the 3-, 4-, and 6-positions of D-mannose The well-known reactivity of concanavalin A with α-D-glucosides and α-D-mannosides provides a precedent for this[32]

Precipitation in anti-Pn XIV, anti-Pn XV, and anti-Pn XVII — Pn S XIV contains residues of D-galactose partly as non-reducing end-groups, partly linked at C-3, C-6, or both, D-glucose, and 2-acetamido-2-deoxy-D-glucose[33]. Again, the cross-reactivity of Ll is difficult to explain unless its content of D-glucose is responsible or unless end-groups of D-glucuronic acid react with antibody determinants accommodated to the few end-groups of D-glucose which occur in S XIV Precipitation by Ls is undoubtedly due to its nonreducing end-groups of D-galactose

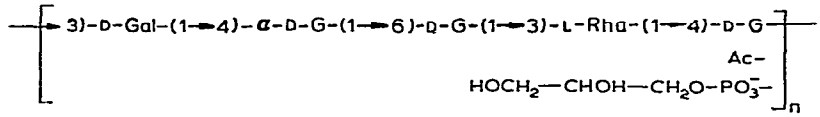
The components found in Pn S XV are galactose, glucose, galactosamine, glucosamine, glycerol, and phosphate groups[31] Similar considerations apply in this instance as were held probable for the reaction in anti-Pn X. The additional possibility that residues of glucuronic acid might fit into antibody spaces designed for glucose is contraindicated by the data in footnote g on prior precipitation with *Aerobacter aerogenes* 418 polysaccharide (K2) which possesses nonreducing end-groups of D-glucuronic acid[34]. Ll precipitated as much antibody from these supernatants as from intact serum

More than one-half of the type-specific antibody in anti-Pn XV was precipitated by Ls, whereas Ll precipitated much less, but a portion of the same fraction of antibody (footnote g) It might be recalled that it was possible to distinguish between

the depyruvylated derivatives of the capsular polysaccharides of two strains of *Rhizobium trifolii*, TA1 and UNZ29, by the large difference in their reactivity[35] in anti-Pn XV.

Pn S XVII consists of galactose, glucose, rhamnose, a polyol, and an unidentified sugar[36] There is a massive cross-reaction in both directions between S XVII and streptococcal group F type IV polysaccharide which contains the same three sugars At least some of the galactose residues in F IV are the D-isomer, occurring as non-reducing end-groups[9] If similar residues occur in S XVII, they would account for the cross-reactivity of Ls in anti-Pn XVII

Precipitation in anti-Pn XVIII — The structure of S XVIII is given as



or its isomer in which the residues of isomaltose and glucose are interchanged An *O*-acetylated sugar is immunodominant[37] Possibly this is responsible for the large cross-reactions in anti-Pn XVIII, particularly as *O*-acetylation seems to be a factor affecting the reactivity of Ls (Table II)

Reactivity in anti-Pn XXII — S XXII contains an as yet unidentified uronic acid[38] and anti-Pn XXII gives precipitates with glycogen and amylopectin[18]

Thus far, the cross-reactivities of the polysaccharides of *Lipomyces lipoferus* and *Lipomyces starkeyi* in anti-Pn II, VII, and XIV have been correlated with what is known of the structures of the substances involved Before this can be said of the often massive cross-precipitations in anti-Pn VIII, X, XV, XVIII, and XXII, additional structural information must be forthcoming for the *Lipomyces* polysaccharides and for S X, S XV, and S XXII To be considered, also, is the possibility that some of the cross-reactions might be caused by undetected impurities in the preparations. This impediment is compensated for, however, by the detection of impurities through such cross-reactions as those presently reported (*cf* Ref 9, type III)

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