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Note

Mammalian-lung galactan

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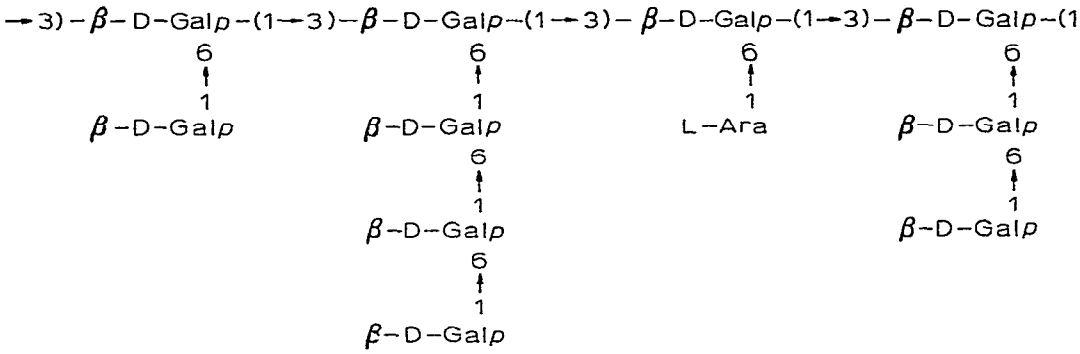
We are involved in the study of six murine IgA myeloma immunoglobulins having activity directed against multiple β-D-(1 → 6)-linked D-galactopyranose residues, namely, proteins[1–4] Jl, S10, X24, X44, T191, and J539. The antigen with which all of these immunoglobulins form a precipitate is a larch arabinogalactan



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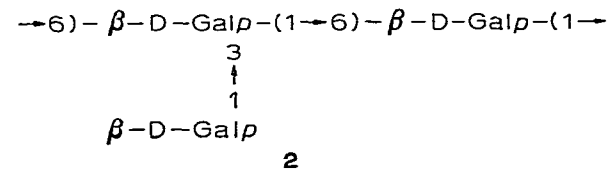
(AG) that is believed[5] to have mainly the structure **1**. We have determined the association constants (*Ka* values) of two of these proteins (X24 and J539) with (1 → 6)-β-D-galactotriose and (1 → 6)-β-D-galactotetraose by fluorescence techniques[3] (*Ka* 1.5–3.5 × 10^5). The results support structure **1** for the arabinogalactan, as the terminal, multiple D-galactopyranosyl side-chain residues would be immunodominant[6]. The *Ka* values are of the same order of magnitude as the one exhibited[7] by the immunodeterminant octasaccharide fragment from *Diplococcus pneumoniae* S-VIII and its homologous rabbit IgG antibody (2.5 × 10^5), and these data strongly suggest that our galactan/antigalactan systems are comparable to a homologous antigen/antibody system.

In 1952, Wolfrom and co-workers proposed structure **2** for beef-lung galactan (LG), based on their methylation studies[8]. Heidelberger and co-workers[9] re-examined the structure of lung galactan by immunochemical methods, and observed that it gave a precipitate with antipneumococcal Type XIV antibody, thus confirming the



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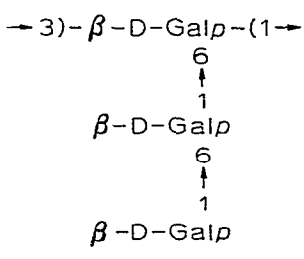
presence of terminal D-galactosyl residues in lung galactan. They also showed that it contains traces of a glucuronic acid. We know that the activity of our myeloma immunoglobulins is directed against multiple β-D-(1 → 6)-linked D-galactopyranosyl residues[1–3]. It is unlikely that (1 → 3) side-chains, such as those in **2**, could be adequately accommodated in the active site of these IgA's for precipitation to occur. It has been proposed[9] that structure **3** would fit the methylation data of Wolfrom *et al.*[8] equally well. In view of our data, such a structure is likely to be closer to the truth, and we propose structure **4** for the following reasons.



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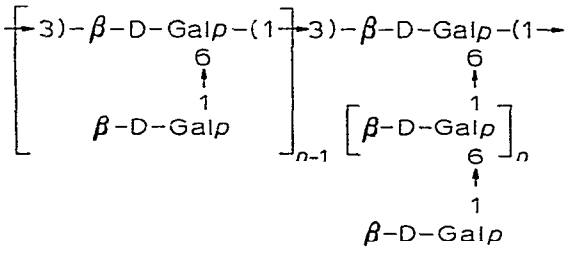

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1. All six of our myeloma IgA immunoglobulins precipitate strongly with the lung galactan (see Fig. 1), showing that the D-galactopyranose residues are β-D-(1 → 6)-linked. It should be noted that Wolfrom *et al.*[8] depicted β-D-linkages in structure **2** without experimental foundation.

2. None of the six anti-galactan myeloma immunoglobulins show a precipitin line with the capsular polysaccharide from *D. pneumoniae* Type XIV (S-XIV) on double diffusion in agar gel, although the S-XIV polysaccharide was used in high concentration (0.4 or 1%). This shows that the presence of single, β-D-(1 → 6)-linked, terminal D-galactosyl residues (as in the repeating unit of S-XIV) is insufficient to cause precipitation with our immunoglobulins. Structure **2** for the lung galactan is, therefore, unlikely.

3. The precipitation of the six IgA myeloma proteins and of lung galactan is poorly inhibited by methyl β-D-galactopyranoside (see Fig. 1) at concentrations at which the precipitation of these IgA's and arabinogalactan (0.1%) is inhibited (not shown).

4. The precipitation of the six anti-galactan IgA's and lung galactan is inhibited by *O*-β-D-galactopyranosyl-(1 → 6)-D-galactose (see Fig. 1).

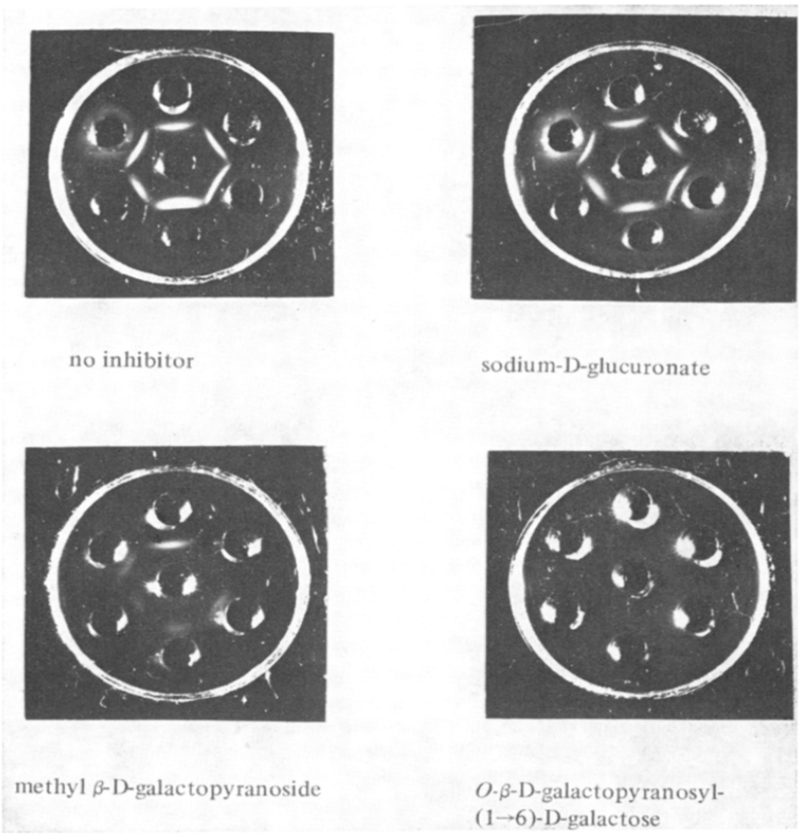

Fig. 1. Agar-gel double-diffusion of IgA immunoglobulins and lung galactan. (Outer wells: clockwise from top: J1, S10, X24, X44, T191, and J539. Inner wells: 0.1% lung galactan. Inhibitors spotted from 0.1M solution. See text and ref. 1 for details.)

The last two points indicate that the lung galactan probably has a structure having the same multiple-residue side-chains of β-D-(1 → 6)-linked D-galactopyranose units as those of the wood arabinogalactan. Furthermore, it may have longer side-chains. The AG polysaccharide may have a lower molecular weight than the LG; this is indicated by the higher rate of diffusion of AG, compared to that of LG, in agar gels (not shown), and this property probably contributes to the difference in precipitation behavior of the two polysaccharides (AG and LG). Another factor affecting the greater precipitation of the LG with our myeloma IgA may be that *long* side-chains of multiple D-galactosyl residues could occur more frequently on the LG backbone than on that of the AG polysaccharide. In that way, *both* active sites of an IgA, 4-chain unit may bind to neighboring side-chains on the same polysaccharide backbone, so that the dimer-IgA becomes a powerful cross-linking agent in the immunoprecipitin lattice; this would be possible for our proposed structure **4**. Relevant to that conclusion is our report[1] that gum ghatti is precipitated by two of these anti-galactan IgA's, yet we have never been able to inhibit this precipitation in

agar gels, not even with (1 → 6)-β-D-galactotetraose. The preceding argument could explain this behavior if it is assumed that gum ghatti has frequent branches of extended multiple, (1 → 6)-β-D-galactose chains, causing *many* IgA dimers to act as crosslinks in the immunoprecipitate. Such a system may be difficult to dissolve with haptens.

The LG polysaccharide may also contain a small proportion of a glucuronic acid[8,9]; if it is present, the type of linkage to its aglycon in the polysaccharide is unknown. Attempted inhibition with sodium D-glucuronate (see Fig. 1) showed no evidence that the uronic acid in LG contributes greatly to the determinant causing precipitation with our anti-galactan IgA's.

EXPERIMENTAL

The lung galactan used was described in ref. 8. Methyl β-D-galactopyranoside was obtained from Pfanstiehl Laboratories, and sodium D-glucuronate from Mann Research Laboratories. *O*-β-D-Galactopyranosyl-(1 → 6)-D-galactose was synthesized by a known procedure[10]. Immunoglobulins were isolated, either as ascites (J1, S10, and T191), or purified from ascites, by affinity chromatography (X24, X44, and J539), as described[2].

The Type XIV pneumococcus polysaccharide (S-XIV) used was either obtained from Squibb (0.4%), or from Dr. B. Prescott of the N.I.H. (0.1 and 1.0%). Both preparations precipitated with H635 horse homologous antibody on agar double-diffusion or in the capillary-precipitin test. Capillary-precipitin tests with J539 or X44 immunoglobulins (both purified by affinity chromatography[2]) and 0.4% S-XIV or 0.1 and 1.0% S-XIV were negative.

Agar gels for Ouchterlony double-diffusion were made up in phosphate-buffered, 0.85% saline at pH 7.4. Lung galactan and mono- and oligo-saccharide solutions were made up in the same buffer. The inhibiting sugar (0.1M) (or buffered saline in the case of the control) was placed in all seven wells of each pattern, which was contained in a circular disc to prevent loss by diffusion. After 1.5 h, the six outer wells were filled with the six different myeloma IgA immunoglobulins, and, after another 1.5 h, the center well was filled with 0.1% beef-lung galactan solution. For details of the agar-gel inhibition method, see ref. 1. Precipitin lines were formed in ~1.5–2 h at room temperature.

ACKNOWLEDGMENTS

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REFERENCES

1 M. Potter, E. Mushinski, and C. P. J. Glaudemans, *J. Immunol.*, 108 (1972) 295.
2 M. Potter and C. P. J. Glaudemans, *Methods Enzymol.*, 28 (1972) 388.
3 M. E. Jolley, S. Rudikoff, M. Potter, and C. P. J. Glaudemans, *Biochemistry*, 12 (1973) 3039.

4 S. RUDIKOFF, M. POTTER, E. MUSHINSKI, C. P. J. GLAUDEMANS, AND M. E. JOLLEY, *J. Exp. Med.*, in press.
5 G. O. ASPINALL, *Polysaccharides*, Pergamon Press, New York, 1970.
6 E. A. KABAT AND M. M. MAYER, *Experimental Immunochemistry*, C. C. THOMAS, Springfield, Mass., 1964.
7 J. SPYER AND A. PAPPENHEIMER, cited in E. HABER, *Fed. Proc.*, 29 (1970) 66.
8 M. L. WOLFROM, G. SUTHERLAND, AND M. SCHLAMOWITZ, *J. Amer. Chem. Soc.*, 74 (1952) 4883.
9 M. HEIDELBERGER, Z. DISCHE, W. B. NEELY, AND M. L. WOLFROM, *J. Amer. Chem. Soc.*, 77 (1955) 3533.
10 K. FREUDENBURG, A. WOLF, E. KNOPF, AND S. H. ZAHEER, *Ber.*, 61 (1928) 1743.