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Note

Further immunochemical studies on the combining sites of *Lotus tetragonolobus* and *Ulex europaeus* I and II lectins

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Plant lectins having well defined, carbohydrate specificities are very useful tools for detection, characterization, and isolation of substances containing carbohydrate side-chains on cell membranes and cell surfaces[1–5]. With respect to lectins isolated from *Lotus tetragonolobus* and *Ulex europaeus* seeds, their carbohydrate specificities have been extensively studied by quantitative precipitin and precipitin-inhibition assays using different mono- and oligo-saccharides[6–8]. Both *Lotus* and *Ulex*-I lectins are H-specific, but their carbohydrate specificities tested by quantitative, precipitin-inhibition assays differed somewhat; with *Lotus* lectin, the most specific oligosaccharide found was an H-active, difucosyl oligosaccharide, JS R_{IM5} 2.5, namely,

α-L-Fuc α-L-Fuc
1 1
↓ ↓
2 3
β-D-Gal-(1→4)-β-D-GlcNAc-(1→6)-3-hexenetetrol,

and with *Ulex*-I lectin, an H-active, monofucosyl oligosaccharide, JS R_L 0.75, namely,

α-L-Fuc
1
↓
2
β-D-Gal-(1→4)-β-D-GlcNAc-(1→6)-3-hexenetetrol[6,7].

Both the *Lotus* and the *Ulex*-I lectin showed relatively high affinity for 2′-*O*-α-L-fucopyranosyllactose[6,7,9]. *Ulex*-II lectin, which shows no blood-group specificity, also

reacted most strongly with the H-active, monofucosyl oligosaccharide JS R_L 0.75 and with 2′-*O*-α-L-fucopyranosyllactose, and also reacted well with β-(1→4)-linked oligomers[8] of D-GlcNAc, as first observed by Matsumoto and Osawa[10]. The specificities of these three lectins for such other, recently synthesized fucopyranosyllactoses[11] as 3′-*O*-α-L-, 3′-*O*-β-L-, and 6′-*O*-α-L-fucopyranosyllactose were not known. It is important to characterize the reactivity of these oligosaccharides relative to those already studied, in order to evaluate the specificity for the α-Fuc-(1→2) structure This has now been achieved by quantitative precipitin-inhibition assays.

EXPERIMENTAL

Plant lectins used in this study were purified by the immunoadsorbent, polyleucyl hog-gastric mucin A + H (ref. 12) according to methods reported previously[6–8,10,13]. Blood-group substances had been prepared in this laboratory[14,15]. Methyl α-L-fucoside was synthesized by Dr. A. Lundblad. L-Fucose and *p*-nitrophenyl α-L-fucoside were purchased from Sigma Chemical Co. Fucopyranosyllactoses were synthesized by reported methods[11]. The β-(1→4)-linked oligomers of D-GlcNAc, such as *N*,*N*′,*N*″-triacetylchitotriose, $(GlcNAc)_3$, and *N*,*N*′,*N*″,*N*‴-tetraacetylchitotetraose, $(GlcNAc)_4$, were kindly provided by Drs. N. Sharon and I. J. Goldstein. Quantitative precipitin assays were performed on a microscale[16], and the nitrogen content of the washed precipitates was determined by the ninhydrin procedure[17].

RESULTS

Fig. 1A shows the abilities of different mono- and oligo-saccharides to inhibit precipitation of *Lotus* lectin by blood-group H substance JS øOH-insoluble. Methyl α-L-Fuc, *p*-nitrophenyl α-L-Fuc, 3′-*O*-β-L-fucopyranosyllactose, and L-Fuc, respectively

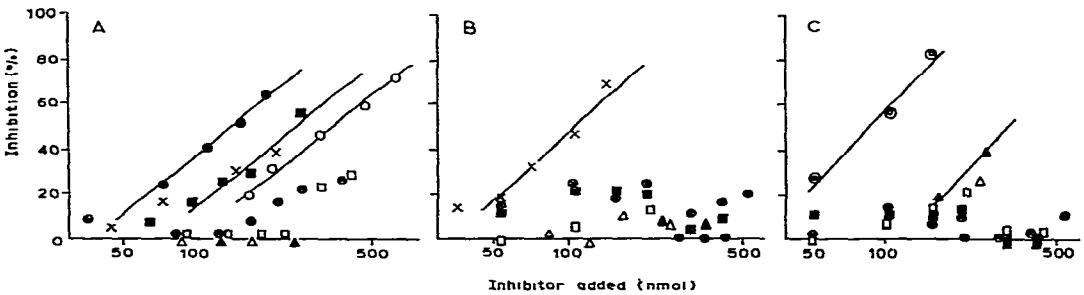

Fig 1. Inhibition, by mono- and oligo-saccharides, of precipitation of 6.7 μg of N of *Lotus* lectin by 32 μg of blood-group H substance JS øOH-insoluble (A), 4 2 μg of N of *Ulex*-I lectin by 18.3 μg of blood-group H substance Tighe øOH-insoluble (B), and 5.2 μg of N of *Ulex*-II lectin by 6.6 μg of blood-group H substance JS øOH-insoluble, 1st IO_4 (C). Inhibitors used were L-Fuc (○), *p*-nitrophenyl α-L-Fuc (X), methyl α-L-Fuc (●), 3′-*O*-α-L-fucopyranosyllactose (□), 3′-*O*-β-L-fucopyranosyllactose (■), 6′-*O*-α-L-fucopyranosyllactose (⊗), $(GlcNAc)_3$ (▲), $(GlcNAc)_4$ (△), and 2′-*O*-α-fucopyranosyllactose (○) Total volume was 250 μL for *Lotus* lectin, 200 μL for *Ulex*-I lectin, and 250 μL for *Ulex*-II lectin

showed 50% inhibition at 135, 240, 240, and 350 nmol; 3'-*O*-α-L-fucopyranosyllactose at 420 nmol, and 6'-*O*-α-L-fucopyranosyllactose at 370 nmol, the highest amounts tested, showed 29 and 26% inhibition, respectively. No inhibition was found with $(GlcNAc)_3$ and $(GlcNAc)_4$ up to 240 nmol.

As shown in Fig. 1B, *p*-nitrophenyl α-L-Fuc at 100 nmol gave 50% inhibition of the precipitate of *Ulex*-I lectin and blood-group H substance Tighe øOH-insoluble. Other glycosides and oligosaccharides tested did not show significant inhibition at amounts up to 500 nmol.

From Fig. 1C, it may be seen that 2'-*O*-α-L-fucopyranosyllactose showed 50% inhibition of precipitation between *Ulex*-II lectin and blood-group H substance JS øOH-insoluble, 1st IO_4; $(GlcNAc)_3$ showed 39% inhibition at 255 nmol (the highest amount used, because of the limited quantity available). Weaker inhibition was observed with the other inhibitors.

The present findings show that, with *Lotus* and *Ulex*-I and II lectins, 3'-*O*-α-L-, 3'-*O*-β-L-, and 6'-*O*-α-L-fucopyranosyllactoses were poorer than the best inhibitors previously studied, namely, methyl α-L-Fuc and JS R_{IM5} 2 5 for *Lotus* lectin, and JS R_L 0.75 for *Ulex*-I and II lectins, respectively[6-8]. These data provide additional evidence of the association of the specificities of these three lectins for an α-L-Fuc-(1→2) structure as a determinant of the blood-group H substance.

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