Studies on the interaction of the mistletoe lectin I with carbohydrates

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Summary. Carbohydrates and modified derivatives of D-galactose have been studied for their capacity to inhibit the hemagglutination of human erythrocytes by mistletoe lectin I. Inhibition data suggest that unmodified hydroxyl groups at the C-2, C-3, and C-4 position of the D-galactopyranosyl ring are essential for binding to the active sites of the lectin.

Using affinity chromatography 3 lectins with mol.wts 115,000, 60,000, and 50,000 were isolated from mistletoe (*Viscum album* L.). The D-galactopyranosyl binding lectin I agglutinates human erythrocytes of all groups and reacts with the carbohydrate moieties of serum proteins[1-3]. It was found that both the agglutination and the reaction of the lectin with glycoproteins could be inhibited by D-galactose. Therefore it was of interest to study quantitatively the lectin-carbohydrate interaction in order to ascertain the configuration features that a molecule must possess in order to bind to the mistletoe lectin.

Material and methods. The lectin I was isolated from plant material (without fruits) of mistletoe, grown on *Robinia pseudoacacia*, by affinity chromatography using acid treated agarose[2]. The hemagglutination inhibition tests were carried out using the microtitrator of Takatsy[4] as follows: to 0.05 ml of a 2-fold serial dilution of a carbohydrate 0.025 ml lectin solution (25 μg per ml phosphate buffered saline pH 7.2) with a hemagglutinating activity of 4 units was added. After incubation at 37 °C for 1 h 0.025 ml of a 2% human erythrocytes suspension was added. After 2 h at room temperature the degree of agglutination was estimated. Stock solutions of 0.2 M carbohydrate in phosphate buffered saline pH 7,2 were prepared and used. Galactan isolated from *Lupinus albus* (β 1-4 linkage) and from *Helix pomatia* (β 1-3, β 1-6 linkage)[5,6] were used as 1% solutions. Washed human erythrocytes group 0 were treated with glutaraldehyde as described by Dalen[7]. Derivatives of D-galactose were prepared by standard methods in our laboratory[8,9].

Inhibition power of various D-galactose derivatives

Inhibiting compound	µmoles carbohydrate/ml needed for complete inhibition of 4 hemagglutination units
D-Galactose	12
Methyl-α-D-galactopyranoside	12
Methyl-β-D-galactopyranoside	6
Phenyl-α-D-galactopyranoside	3
Phenyl-β-D-galactopyranoside	1.5
Lactulose (β 1-4)	3
Lactose (β 1-4)	3
Melibiose (α 1-6)	12
Raffinose (α 1-6, β 1-2)	12
2-Deoxy-D-galactose	> 200 (0)
3-O-Methyl-D-galactose	> 200 (0)
3,6-Anhydro-D-galactose	> 200 (0)
4-O-Methyl-D-galactose	> 200 (0)
4,6-O-Ethylidene-D-galactose	> 200 (0)
4,6-O-Benzylidene-D-galactose	> 200 (0)
1,2,3,4-O-Diisopropylidene-D-galactose	> 200 (0)
6-Deoxy-D-galactose	12
6-O-Methyl-D-galactose	12
6-O-D-Benzyl-D-galactose	3
Galactan from *Lupinus albus* (β 1-4)	> 10 mg (0)
Galactan from *Helix pomatia* (β 1-3; β 1-6)	> 10 mg (0)

Where numbers are given in parentheses, inhibition was not attained with 0.2 M carbohydrate solution.

Results and discussion. Our inhibition experiments confirmed the original observation of Pardoe[10], made with crude extracts of mistletoe, that D-galactose and lactose are good inhibitors of the lectin's hemagglutinating activity. We extended the study to some other carbohydrates and found no inhibition by the following carbohydrates at 0.2 M concentrations: D-glucose, D-glucosamine, N-acetyl-D-glucosamine, D-mannose, D-mannosamine, N-acetyl-D-mannosamine, D-galactosamine, N-acetyl-D-galactosamine, D-talose, D-tagatose, galactitol, maltose, and sucrose. Among inhibiting D-galactosides differing only in the configuration about anomeric C-1 there seems to be a slight preference for the β anomer. Aromatic aglycons of D-galactosides increased binding of the corresponding sugars by the lectin. This does not exclude the existence of a hydrophobic region adjacent to the sugar binding site (table). In this respect mistletoe lectin I is similar to concanavalin A and soy bean agglutinin[11,12]. The fact that N-acetyl-D-galactosamine, D-galactosamine, 2-deoxy-D-galactose, D-talose, and D-tagatose did not inhibit indicates a requirement for a free equatorial hydroxyl group at C-2 in order to bind the lectin. The same applies to the configuration at C-3 because neither 3-O-methyl-D-galactose, 3,6 anhydro-D-galactose, nor galactan from the snail *Helix pomatia* (β 1-3, β 1-6 linkage) were inhibitors. Also at C-4 an equatorial hydroxyl group is necessary for the lectin-carbohydrate interaction since D-glucose, 4-O-methyl-D-galatose, 4,6-O-ethylidene-D-galactose, 4,6-O-benzylidene-D-galactose and galactan from *Lupinus albus* (β 1-4 linkage) did not inhibit hemagglutination. But a C-6 hydroxyl group seems not to be essential for binding, as 6-deoxy-D-galactose, 6-O-methyl-D-galactose, and 6-O-benzyl-D-galactose were inhibitors. These inhibition data demonstrate that substitution of any of the hydroxyl groups at C-2, C-3 and C-4 results in a complete loss of inhibiting activity. Therefore unmodified hydroxyl groups at the position C-2, C-3, and C-4 of the D-galactopyranosyl ring system are essential for the carbohydrate-lectin interaction. Similar results have been reported for the lectin from *Ricinus communis*, although these experiments were performed with a crude lectin preparation[13].

1 H. Franz, B. Haustein, P. Luther, U. Kuropka and A. Kindt, Acta biol. med. germ. *36*, 113 (1977).
2 P. Ziska, H. Franz and A. Kindt, Experientia *34*, 123 (1978).
3 P. Ziska and H. Franz, Experientia *35*, 161 (1979).
4 G. Takatsy, Symp. Ser. Immunbiol. Stand. *4*, 275 (1967).
5 J.K.N. Jones and Y. Tanaka, Meth. Carbohydr. Chem. *5*, 132 (1965).
6 H.J. Horstmann, Biochem. Z. *340*, 548 (1964).
7 A.B. Dalen, Acta path. microbiol. scand. B *84*, 196 (1976).
8 D.J. Bell, Adv. Carbohydr. Chem. *6*, 11 (1951).
9 G.G. Mahler, Adv. Carbohydr. Chem. *10*, 273 (1955).
10 G.I. Pardoe, G.W. Bird, G. Uhlenbruck, I. Sprenger and M. Heggen, Z. Immunfschg. *140*, 374 (1970).
11 R.P. Poretz and I.J. Goldstein, Biochem. Pharamc. *20*, 2727 (1971).
12 M.E. A. Pereira, E. Kabat and N. Sharon, Carbohydr. Res. *37*, 89 (1974).
13 R.G. Drysdale, P.R. Herrick and D. Franks, Vox Sang. *15*, 194 (1968).