Mannose-specific plant lectins from plants as diagnostics, vaccines and tools for the elucidation of viral infection mechanisms in animals

E. Davidson, J.M.S. Forrest, I.M. Morrison & D. Stewart

Lectins are proteins which bind carbohydrates both specifically and reversibly. One class of these proteins, mannose-specific lectins (MSLs), binds mannose (Man) residues. Mannose is an abundant cell surface sugar present in glycoconjugates that are implicated in a wide range of important receptor-mediated cellular processes. The MSLs, therefore, are potentially useful tools in research, with a wide scope of potential applications. Daffodil (*Narcissus pseudonarcissus* agglutinin, NPA) and snowdrop (*Galanthus nivalis* agglutinin, GNA) lectins were the first shown to exhibit this exclusive mannose specificity. Additionally, they demonstrated a more complex specificity, than for example Con A, in that they distinguished Man-Man linkages, with NPA preferring Man α(1-6) Man and GNA favouring Man α(1-3) Man linkages (Fig. 1).

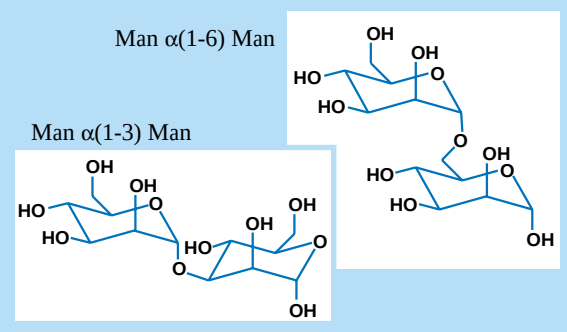

Figure 1 Man α(1-3)Man and Manα(1-6)Man linkages.

MSLs have been found to bind to the mannosylated region of the envelope glycoproteins of various retroviruses such as human (HIV), simian (SIV) and feline (FIV) immunodeficiency viruses. These glycoproteins are the site of interaction with the cell surface molecule CD4, the co-receptor for HIV virus. HIV binds to CD4 *via* an interaction between the first domain of CD4 and a discontinuous region of the HIV-1 outer envelope glycoprotein gp120. Similar interactions have been implicated in SIV and FIV pathogenesis. Binding of selected MSLs to the envelope glycoprotein of HIV viruses has resulted in the potent and selective inhibition of infection *in vitro*[1].

The MSLs were initially thought to be limited to only a few species but our studies have shown that they are present, albeit often at very low levels, in a wide variety of cultivated agricultural and horticultural crops.

Linkage specificity Although all the MSLs were purified on a mannose-affinity column and were shown to agglutinate rabbit red blood cells, thereby indicating their specificity for mannose, their detailed specificity remained undefined. This was remedied by employing quantitative precipitation and precipitation-inhibition assays. Highly branched mannan from the yeast *S. cerevisiae* was used to quantitatively precipitate the lectin. Selected oligomannans, with defined mannose-mannose linkages, were purified to homogeneity by reverse-phase HPLC and used to inhibit the precipitation of the MSL/mannan system. The concentration of oligomannan required to obtain a 50% inhibition of precipitation was determined and a relative inhibitory potency (RIP) ranking was established with D-mannose ranked at 1 (Table 1).

Both NPA and GNA exhibited their reported linkage preferences of α(1-6) and α(1-3), respectively. The presence of a reducing terminal did not make a significant difference to their relative inhibitory potency. For example, in the assays of GNA, methylation of the reducing terminus of

Lectin	Snowdrop (GNA)	Daffodil (NPA)	Amaryllis (HHA)	Ramson (AUA)	Shallot (AAA)
M. Wt. (kD)	12.5	12.5	12.5	12.5	12.5
D-Man	1.0	1.0	1.0	1.0	1.0
α-D-Man p-OMe	1.8	1.7	1.3	1.7	1.2
β-D-Man p-OMe	0.1	0.3	0.7	0.3	0.6
Man β(1-2) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man β(1-2) Man β(1-2) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man β(1-4) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man β(1-4) Man β(1-4) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man α(1-2) Man	2.2	2.9	3.2	3.6	3.4
Man α(1-3) Man	12.1	3.8	3.7	5.3	6.1
Man α(1-4) Man	1.1	1.7	1.4	1.3	1.9
Man α(1-6) Man	3.2	5.0	6.0	4.1	17.0
Man α(1-3) Man α-OMe	11.7	4.8	10.5	10.2	10.9
Man α(1-6) Man α-OMe	3.5	6.0	12.3	5.1	9.0
Man α(1-6) Glc	2.3	2.0	4.4	4.2	1.9
Man α(1-6) Man α(1-6) Man	2.5	6.7	22.7	7.0	9.0
Man α(1-2) Man α(1-3) / Man α(1-2) Man α(1-6) }Man-R	2.1	0.9	4.4	2.2	2.0
Man α(1-4) / Man α(1-2) }Man-OMe	1.9	2.3	1.7	2.4	2.2
Man α(1-3) / Man α(1-6) }Man-OMe	22.1	5.2	14.2	11.3	17.0
M α(1-6) / M α(1-3) }M α(1-6) / M α(1-3) } M β(1-4) GlcR R = NAc β(1-4) Glc NAC-Asn	28.0	30.1	33.2	30.0	27.1
M α(1-6) / M α(1-3) }M α(1-6) / Man α(1-2) Man α(1-3) }M β(1-4) GlcR R = NAc β(1-4) Glc NAC-Asn	30.1	28.3	47.3	35.0	21.8
Linkage preference	α(1-3)	α(1-6)	α(1-6)>(1-3)	α(1-3)>(1-6)	α(1-3)>(1-6)

Table 1 The mannose binding preferences of selected mannose-specific lectins. The concentrations of oligomannan (mM) required to obtain a 50% inhibition of precipitation was determined and a relative inhibitory potency (RIP) ranking was established with D-mannose ranked at 1.

Man α(1-3) Man only changed the RIP from 12.1 to 11.7. Similarly, for NPA the methylation of Man α(1-6) Man changed the RIP from 5.0 to 6.0.

The linkage preference was less clearly defined for the other lectins. The amaryllis MSL, (*Hippeastrum hybrid* agglutin; HHA) exhibited a slight preference of α(1-6) over α(1-3) linkages. The most significant difference was the preferential binding of non-terminal Man-Man linkages. For example, methylation of the reducing terminus of Man α(1-3) Man and Man α(1-6) Man changed the RIP values from 3.7 and 6.0 to 10.5 and 12.3, respectively. This preference for internal linkages is reflected in the high RIP values (33.2 and 47.3) obtained with the complex oligomannosaccharides.

The ramson lectin (*Allium ursinum* agglutin; AUA) displayed a linkage preference similar to snowdrop (GNA) but with a greater affinity for the internal α(1-3) linkage. Methylation of the reducing terminal of Man α(1-3) Man resulted in an almost 100% increase in RIP value for AUA.

The shallot lectin (*Allium ascalonicum* agglutin; AAA) exhibits unusual linkage specificities with the RIP values suggesting that terminal α(1-6) and internal α(1-3) linkages were favoured. Significantly, the RIP values for the complex oligomannosaccharides were the lowest of the MSLs shown, possibly indicating that this lectin favours smaller substrates.

Isolectin distribution and activity *In planta*, the lectins are often the product of more than one gene leading to the production of iso-lectins, proteins which vary slightly in their amino acid composition and hence net charge. This can lead to variation in

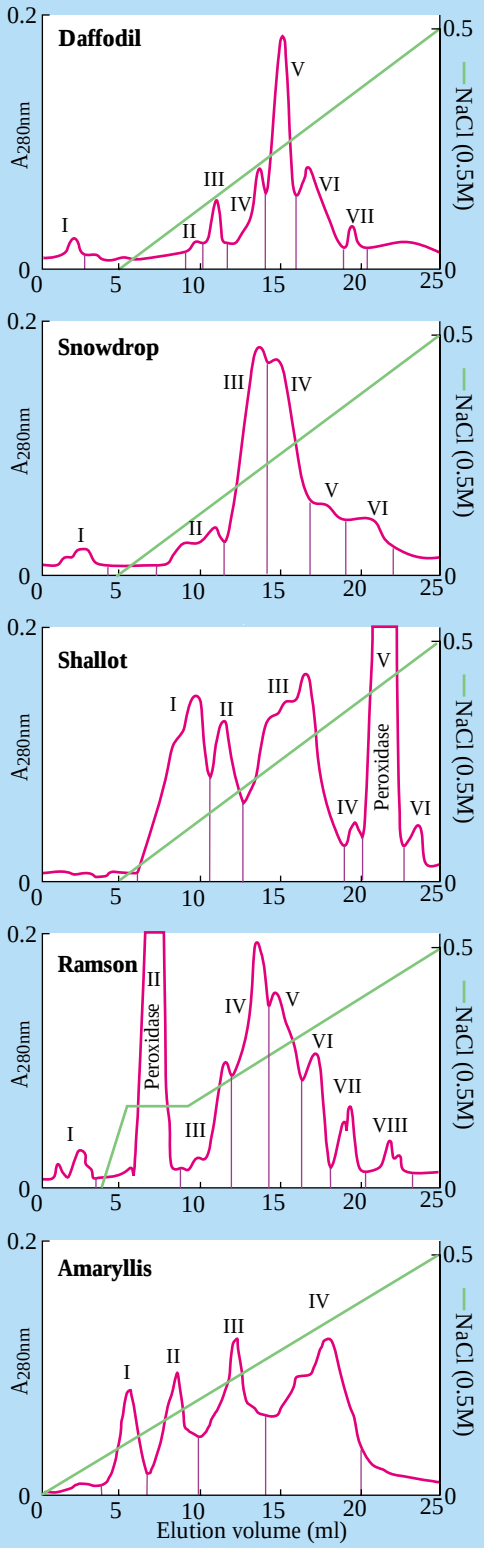

Figure 2 HPLC chromatographs of selected mannose-specific lectins.

Lectin	Agg.	gp120	gp148
Daffodil			
I	-	-	-
II	-	-	-
III	+	+	+
IV	+	+	+
V	++	++	++
VI	+	+	+
VII	-	-	-
Ramson			
I	-	-	-
II	-	-	-
III	+	-	-
IV	++	++	++
V	++	++	+
VI	+	+	-
VII	+	+	-
VIII	-	-	-
Snowdrop			
I	-	-	-
II	-	-	-
III	++	++	++
IV	++	+	++
V	+	+	+
VI	-	-	-
Shallot			
I	++	+	++
II	++	+	+
III	++	++	+
IV	-	-	-
V	-	-	-
Amaryllis			
I	+	+ +	+ +
II	+	++	++
III	+	++	++
IV	++	+	+

+, ++ mild and intense binding/agglutination
- no binding/agglutination

Table 2 The (rabbit) red blood cell agglutinating (Agg.) and HIV(gp 120) and SIV (gp 148) binding abilities of the fractions obtained from the IEHPLC of selected mannose-specific lectins.

the 'saccharide'-binding site. To study this, selected MSLs were subject to ion exchange HPLC to separate any isolectins and test them for agglutination and HIV and SIV binding activities (Fig. 2).

The iso-lectin profiles were distinct giving a disparate range of binding activities. However, there were no cases of immunodeficiency virus binding activity without agglutination activity, which supports the premise that the isolates are actually binding to the immunodeficiency viruses *via* the complex mannan side chains.

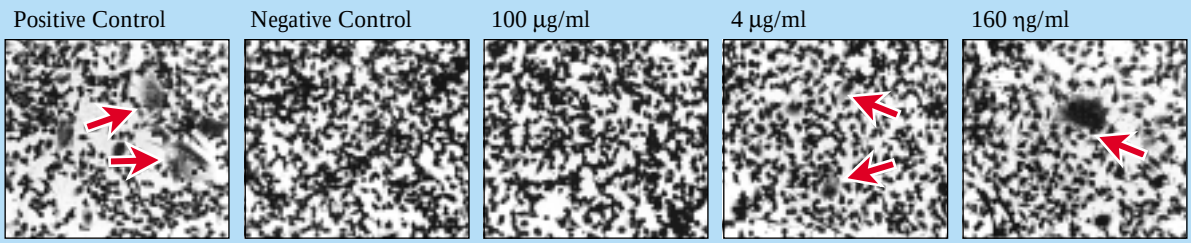

Figure 3 Inhibition of FIV infection by selected MSLs. Arrows indicate syncytia formation.

Some of the MSL fractions displayed distinct HIV and SIV binding. The chromatogram of the shallot, AAA, showed that optimal HIV and SIV binding activities were separated and found in fractions III and I, respectively. This separation of activities suggests that the substrate specificities of the isolectins are different, a fact highlighted during analysis of the linkage specificities (Table 1).

In vivo anti-immunodeficiency virus (IV) activity In conjunction with Drs Brian Willett and Margaret Hosie (Faculty of Veterinary Medicine, Retrovirus Research Laboratory, University of Glasgow), selected MSLs (Table 1) were used to study the efficacy of such lectins at inhibiting the *in vivo* infection of FIV, an HIV model system. These all exhibited, to varying extents, a dose-dependent inhibition of FIV infection. Only the infected cells exhibited the expected syncytia formation. The lectin itself did not induce syncytia formation. The dose-dependency of lectin inhibition was reflected in the reduced incidence of infection-related syncyitia formation accompanying an increase in lectin concentration (Fig. 3).

An ELISA based on production of the FIV protein p24, showed that HHA proved to be the most potent inhibitor of FIV infection (Fig. 4). This gave an 85% reduction in viral p24 production at a lectin concentration of 160 ng/ml. GNA and NPA also performed well, exhibiting 76% and 69% inhibition, respectively, at this concentration. These results reflect previous findings for HIV[1] and highlight the benefits of using the FIV animal model system.

Conclusion Plant-derived mannose specific lectins are clearly more prevalent than was first thought. Although simply classed as mannose specific, there are a broad range of linkage specificities and affinities within this class of lectins. It is evident, from HPLC analysis alone, that the majority of the existing and novel MSLs exist as a collection of iso-lectins and that these exhibit distinct and often varied specificities and affinities towards simple and complex substrates.

The ability of the MSLs to bind immunodeficiency viruses means that they represent an academic and economic opportunity, hitherto untapped. Academically, they will allow the part played by the mannosylated regions of IV in the infection process to be elucidated. Economically, they have the potential for development as ELISA-based IV-detection systems or, by employing anti-idiotypic antibody methodology, they offer the chance to produce IV vaccines.

References

[1] Weiler, B.E, Schröder, H.C., Stefanovich, V., Stewart, D., Forrest, J.M.S., Allen, L.B., Bowden, B.J., Kreuter, M.H., Voth, R. & Müller, W.E.G. (1990). *Journal of General Virology* 71, 1957-1963.

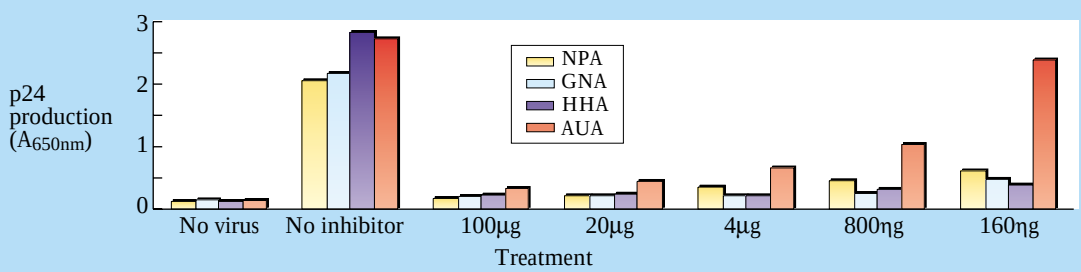

Figure 4 Inhibition of FIV infection by MSLs from daffodil (NPA), snowdrop (GNA), amaryllis (HHA) and ramson (AUA). Inhibition of virus replication was reflected in reduced production of the viral protein p24.