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New mannose-specific lectins from garlic (*Allium sativum*) and ramsons (*Allium ursinum*) bulbs *

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ABSTRACT

Two new mannose-binding lectins were isolated from garlic (*Allium sativum*, ASA) and ramsons (*Allium ursinum*, AUA) bulbs, of the family Alliaceae, by affinity chromatography on immobilized mannose. The carbohydrate-binding specificity of these two lectins was studied by quantitative precipitation and hapten-inhibition assay. ASA reacted strongly with a synthetic linear (1 → 3)-α-D-mannan and *S. cerevisiae* mannan, weakly with a synthetic (1 → 6)-α-D-mannan, and failed to precipitate with galactomannans from *T. gropengiesseri* and *T. lactis-condensi*, a linear mannopentaose, and murine IgM. On the other hand, AUA gave a strong reaction of precipitation with murine IgM, and good reactions with *S. cerevisiae* mannan and both synthetic linear mannans, suggesting that the two lectins have somewhat different binding specificities for α-D-mannosyl units. Of the saccharides tested as inhibitors of precipitation, those with α-(1 → 3)-linked mannosyl units were the best inhibitors of ASA, the α-(1 → 2)-, α-(1 → 4)-, and α-(1 → 6)-linked mannobioses and biosides having less than one eighth the affinity of the α-(1 → 3)-linked compounds. The *N*-terminal amino acid sequence of ASA exhibits 79% homology with that of AUA, and moderately high homology (53%) with that of snowdrop bulb lectin, also an α-D-mannosyl-binding lectin.

INTRODUCTION

Recently, we characterized the detailed carbohydrate-binding specificity of a new class of mannose-binding lectins isolated from snowdrop, daffodil, and amaryllis bulbs of the family Amaryllidaceae. These lectins possess high specificity for D-mannose, but differ somewhat from each other in their interaction with manno-oligosaccharides[1–5].

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In the present paper, we describe the sugar-binding specificity of two new mannose-specific lectins from garlic (ASA) and ramsons (AUA) bulbs. These plants belong to the Alliaceae family, which is of the same order, Liliales, as the family Amaryllidaceae[6]. We also compare the sugar-binding specificity of ASA and AUA with the three Amaryllidaceae lectins.

RESULTS AND DISCUSSION

The bulb lectin from garlic (*Allium sativum*, ASA) reacted strongly with a synthetic linear (1 → 3)-α-D-mannan, moderately with a mannan from *S. cerevisiae*, and only slightly with a synthetic linear (1 → 6)-α-D-mannan (Fig. 1). On the other hand, ASA failed to react with *P. pastoris* mannan, *T. gropengiesseri* and *T. lactis-condensi* galactomannans, Man–BSA conjugate, linear mannopentaose, synthetic linear (1 → 3)-α-D-glucan, bovine IgG, and rat and murine IgM (Fig. 1).

Conversely, the bulb lectin from ramsons (*Allium ursinum*, AUA) gave a pronounced precipitation reaction with murine IgM; this lectin also reacted well with the synthetic linear (1 → 3)- and (1 → 6)-α-D-mannans and the *S. cerevisiae* mannan (Fig. 2), but only slightly with the galactomannan from *T. lactis-condensi*. However AUA did not react with the Man–BSA conjugate, *C. lipolytica* galactomannan, glycogen, dextran, pullulan, and bovine IgG. These observations suggest that the binding specificity of AUA is quite different from that of ASA, especially in its interaction with murine IgM. In this regard we have already studied the interaction of murine IgM with the mannose-binding bulb lectins from snowdrop (GNA), daffodil (NPA), and amaryllis (HHA). ASA is most similar to the snowdrop bulb lectin (GNA)[1], with the exception that GNA gave a good precipitation reaction with the synthetic linear (1 → 6)-α-D-mannan but interacted only slightly in a precipitation assay with murine IgM despite its ability, in its immobilized form,

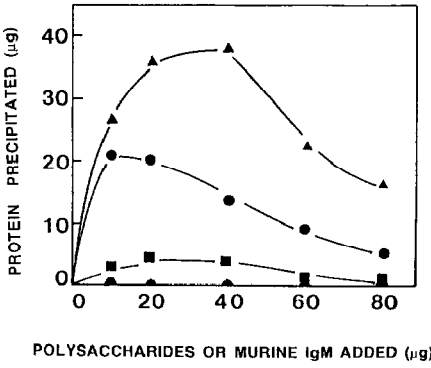

Fig. 1. Quantitative precipitation of mannans by ASA. Each tube contained 30 μg of ASA. Symbols: ▲, synthetic linear (1 → 3)-α-mannan; ●, *S. cerevisiae* mannan; ■, synthetic linear (1 → 6)-α-mannan; ⬤, murine IgM. ASA did not form precipitates with Man–BSA conjugate, *P. pastoris* mannan, *T. gropengiesseri* galactomannan, mannopentaose, (1 → 3)-α-glucan, and rat and bovine IgG.

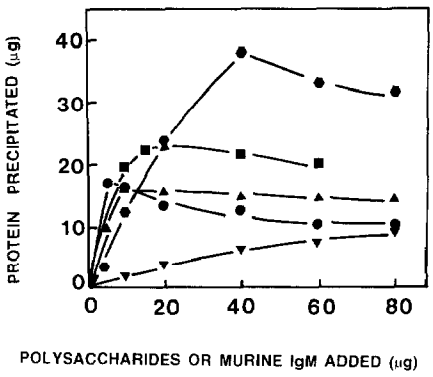

Fig. 2. Quantitative precipitation of mannans and galactomannan by AUA. Each tube contained 20 μg of AUA. Symbols: ●, murine IgM; ■, synthetic linear (1 → 6)-α-mannan; ▲, synthetic linear (1 → 3)-α-mannan; •, *S. cerevisiae* mannan; ▼, *T. lactis-condensi* galactomannan. AUA did not react with Man–BSA conjugate, *C. lipolytica* galactomannan, glycogen, dextran, pullulan, or bovine IgG.

to bind murine IgM[7]. On the other hand, AUA is closely similar to the HHA lectin[3] in its carbohydrate-binding activity.

Of the monosaccharides tested as inhibitors of ASA–mannan precipitation, D-mannose had a higher affinity for ASA than methyl α- or β-D-glucoside or

TABLE I

Inhibition by saccharides of mannan precipitation by ASA and AUA

Sugar	Concentration, mM (degree of inhibition, %)			
	ASA		AUA	
D-Man	40.5	(50)	72	(50)
α-D-Man*p*-OMe	27	(50)	56	(50)
β-D-Man*p*-OMe	80	(0)		
α-D-Man*p*-O-*p*-NP [a]	10	(33)		
β-D-Man*p*-O-*p*-NP	10	(0)		
2-Deoxy-D-Man [b]	150	(15)		
3-Deoxy-D-Man [b]	70	(50)		
6-Deoxy-D-Man [c]	100	(34)		
α-D-Glc*p*-OMe	100	(13)		
β-D-Glc*p*-OMe	100	(9)		
α-D-Glc*p*NAc-OMe	100	(22)		
Manα1-2Man	20	(38)	20	(33)
Manα1-3Man	3.5	(50)	10	(30)
Manα1-3Manα-OMe	3.4	(50)	7	(45)
Manα1-3Manα-O-allyl	3.5	(50)	10	(31)
Manα1-4Manα-OMe	20	(30)	20	(0)
Manα1-6Manα-OMe	20	(14)	14	(50)
Manα1-6Glc	10	(0)		
Manα1-6Manα1-6Man			10.4	(50)
Man_4Man-ol	3	(30)		
Manα1-6 Manα1-3 > Manα-OMe	8	(28)	6.4	(50)

[a] *p*-NP = *p*-nitrophenyl. [b] 2-Deoxy-, and 3-deoxy-D-*arabino*-hexose, respectively. [c] D-Rhamnose.

methyl 2-acetamido-2-deoxy-α-D-glucoside (9% to 22% inhibition at 100 mM Table I). This lectin prefers the α configuration: for α-D-Man*p*-OMe a concentration 27 mM was required for 50% inhibition of the ASA system, whereas β-D-Man*p*-OMe at 80 mM gave no inhibition. Deoxy derivatives (2-deoxy-D-Man, 3-deoxy-D-Man, and 6-deoxy-D-Man, see Table I) were also much less active than D-mannose.

Of the oligosaccharides examined, (1 → 3)-α-mannobiose and its derivatives (Manα1-3Manα-OMe and Manα1-3Manα-O-allyl) were the best inhibitors of the ASA–mannan system, concentrations of 3.5 mM being required for 50% inhibition. It appears that the α-(1 → 3) linkage is most complementary to the sugar-binding site of ASA [(1 → 2)-, (1 → 3)-, and (1 → 6)-α-mannobiose and their glycosides were poorer inhibitors]. Interestingly, mannotetraosylmannitol (Manα1-3 Manα1-3Manα1-3Manα1-2Man-ol, Man_4Man-ol) was less active than (1 → 3)-α-mannobiose, suggesting that, in some manner, an extension of α-(1 → 3)-linked mannose units inhibits binding to ASA. Furthermore, maltose [(1 → 4)-α-glucobiose] and nigerose [(1 → 3)-α-glucobiose] were noninhibitors of the precipitation reaction. Interestingly, the branched mannotrisaccharide Manα1-3(Manα1-6) Manα-OMe was less inhibitory than methyl (1 → 3)-αmannobioside. This observation indicates that the mannose residue attached at the C-6 position of the penultimate mannosyl unit may exert steric hindrance on ASA sugar-binding sites.

These observations indicate that the binding specificity of ASA differs from those of the three aforementioned mannose-binding lectins (GNA, NPA, and HHA) in its low affinity for methyl (1 → 6)-α-mannobioside (Table II). It appears that the sugar-binding sites of ASA are complementary to α-(1 → 3)-linked mannosyl units. Thus, ASA appears to be more closely related in its carbohydrate-bind-

TABLE II

Inhibition by various sugars of five mannan-specific lectin–yeast mannan precipitation systems

Sugar	Relative inhibitory potency				
	ASA	AUA	GNA[a]	NPA[b]	HHA[b]
D-Man	1.0	1.0	1.0	1.0	1.0
α-D-Man*p*-OMe	1.5	1.3	1.6	1.2	1.5
β-D-Man*p*-OMe	≪ 0.4		0.3	0.2	0.5
Manα1-2Man	< 1.4	< 3.6	2.1	3.3	3.2
Manα1-3Man	11.5	< 7.2	12.1	2.8	5.9
Manα1-3Manα-OMe	11.9	10	14.2	3.1	10.5
Manα1-3Manα-O-allyl	11.5	< 7.2			
Manα1-4Manα-OMe	< 1.4	≪ 3.2	1.9	< 0.7	< 2.0
Manα1-6Manα-OMe	≪ 1.4	5.1	4.3	5.1	8.3
Manα1-6Manα1-6Man		6.9	1.9	0.7	20.0
Manα1-6 / Manα1-3 > Manα-OMe	≪ 3.6	11.3	28.3	3.8	13.8

[a] Data from Shibuya et al.[1] using the GNA–*H. capsulata* mannan precipitation system. [b] Data from Kaku et al.[3] using the NPA– and HHA–*P. pastoris* mannan precipitation systems.

	1										11									
ASA	R	N	I	L	M	N	G	E	G	L	Y	A	G	Q	S	L	D	V	E	P
AUA	R	N	I	L	G	N	G	E	G	L	Y	A	G	Q	S	L	E	E	G	P
GNA	D	N	I	L	Y	S	G	E	T	L	S	T	G	E	F	L	N	Y	G	S

	21										31					
ASA	Y	H	F	I	M	Q	E	D	C	N						
AUA	Y	R	L	I	M	Q	E	D		N	L	V	L	Y	E	
GNA	F	V	F	I	M	Q	E	D	C	N	L	V	L	Y	D	

Fig. 3. *N*-Terminal amino acid sequences of ASA, AUA, and GNA, described by the standard one-letter code[15].

ing specificity to GNA, with the exception that (1 → 3)-α-mannobiose or bioside is only a three times better inhibitor than methyl (1 → 6)-α-mannobioside for the GNA precipitation system, and GNA has a very strong affinity for the branched mannotrioside Manα1-3(Manα1-6)Manα-OMe.

On the other hand, Manα1-3(Manα1-6)Manα-OMe was the best inhibitor of the AUA–mannan precipitation system. This branched mannotrisaccharide was 1.6 times better than the α-(1 → 6)-linked mannobioside and exhibited inhibitory potency similar to that of methyl (1 → 3)-α-mannobioside. Thus, the sugar-binding specificity of AUA is similar to that of HHA (Table II). Moreover, the major structure of the high-mannose type oligosaccharide unit of murine IgM is $Man_6GlcNAc$ (ref. 8). We already noted that $Man_5GlcNAc_2Asn$ (GP-V) bound to an HHA-Sepharose column and $Man_6GlcNAc_2Asn$ showed even greater affinity for this column. This observation also indicates that AUA should react with murine IgM.

It has already been noted that the bulb lectins from the Alliaceae and Amaryllidaceae are related serologically[6] and exhibit high affinity for D-mannose. The amino acid compositions of ASA and AUA are very similar[6], and the *N*-terminal amino acid sequences of ASA and AUA, presented in Fig. 3, indicate 79% homology between these two lectins. Furthermore, the amino acid sequence of ASA shows a rather high homology (53%) with that of GNA.

In summary, new mannose-binding lectins have been isolated from garlic and ramsons bulbs. These two lectins exhibit subtle differences from each other in their affinity for α-D-mannosyl units, and also from the three mannose-binding bulb lectins described previously (Table II). We believe these fine differences in sugar-binding specificity should make these bulb lectins useful tools for investigating saccharides and glycoconjugates containing α-D-mannosyl units.

EXPERIMENTAL

The garlic and ramsons lectins (ASA and AUA, respectively) were isolated from extracts of their bulbs by affinity chromatography on immobilized mannose as previously reported[6].

Saccharides and glycoproteins. — Manα1-3Man and Manα1-3Manα-O-allyl were the gifts of Dr. K.L. Matta of the Roswell Park Memorial Institute, Buffalo, NY. Manα1-3Manα-OMe, Manα1-4Manα-OMe, and Manα1-6Manα-OMe were purchased from the Sigma Chemical Co., St. Louis, MO. The synthetic phosphorylated mannopentaose (P-Manα1-3Manα1-3Manα1-3Manα1-2Man) was the generous gift of Dr. Y.C. Lee, The Johns Hopkins University, Baltimore, MD. Mannopentaose was prepared from phosphorylated mannopentaose by treatment with alkaline phosphatase (Type XXX from unweaned-calf intestinal mucosa, Sigma), and Man_4Man-ol was prepared from mannopentaose by reduction with sodium borohydride as previously described[5].

Murine IgM and IgG were obtained from Dr. J.L. Claflin of the University of Michigan, and bovine and rat IgG were purchased from the Sigma Chemical Co. Yeast mannans and galactomannans from various strains were supplied by Dr. P.A.J. Gorin of the Universidade Federal do Parana, Curitiba, Brazil. A synthetic linear (1 → 6)-α-D-mannan, a (1 → 3)-α-D-mannan (dp 30) and (1 → 3)-α-D-glucan were generously provided by Dr. C. Schuerch, State University of New York at Syracuse[9–11]. Other monosaccharides and their derivatives were commercially available.

Quantitative precipitation and precipitation-inhibition assays. — Quantitative precipitation assays were carried out by a micro technique[12]. ASA (30 μg or AUA (20 μg) was mixed with varying amounts of polysaccharides, manno-oligosaccharides, or immunoglobulins in a total volume of 110 or 150 μL (respectively) of 10 mM phosphate buffer (pH 7.2) containing 0.1 mM $CaCl_2$, 0.04% NaN_3, and 150 mM NaCl. After incubation at 37° for 1 h, the mixtures were kept at 4° for 2 days, and centrifuged at 8800*g* for 10 min. Protein in the precipitates was determined by the method of Lowry et al.[13], using bovine serum albumin as standard.

Sugar inhibition of the precipitation reactions was carried out by adding increasing amounts of sugar or sugar derivative to precipitation systems containing lectin and *S. cerevisiae* mannan. The precipitated protein was determined by the procedure of Lowry et al.[13]

Amino acid sequencing. — Amino acid sequencing was performed by Drs. S.F. Perini and P. Andrews of the University of Michigan Biomedical Core Facility[14].

REFERENCES

1 N. Shibuya, I.J. Goldstein, E.J.M. Van Damme, and W.J. Peumans, *J. Biol. Chem.*, 263 (1988) 728–734.
2 H. Kaku and I.J. Goldstein, *Methods Enzymol.*, 179 (1989) 327–331.
3 H. Kaku, E.J.M. Van Damme, W.J. Peumans, and I.J. Goldstein, *Arch. Biochem. Biophys.*, 279 (1990) 298–304.
4 H. Kaku, I.J. Goldstein and S. Oscarson, *Carbohydr. Res.*, 213 (1991) 109–116.
5 H. Kaku and I.J. Goldstein, *Carbohydr. Res.*, 229 (1992) 337–346.
6 E.J.M. Van Damme, I.J. Goldstein, and W.J. Peumans, *Phytochemistry*, 30 (1991) 309–514.
7 N. Shibuya, J.E. Berry, and I.J. Goldstein, *Arch. Biochem. Biophys.*, 267 (1988) 676–680.

8 R. Brenckle and R. Kornfeld, *Arch. Biochem. Biophys.*, 201 (1980) 160–173.
9 J.W.-P. Lin and C. Schuerch, *J. Polym. Sci., Part A-1,* 10 (1972) 2045-2060.
10 F. Kong and C. Schuerch, *Macromolecules*, 17 (1984) 983–989.
11 F.J. Good, Jr. and C. Schuerch, *Macromolecules*, 18 (1985) 595–599.
12 L.L. So and I.J. Goldstein, *J. Biol. Chem.*, 242 (1967) 1617-1622.
13 O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.
14 D.R. Koo, E.T. Morgan, G.E. Tarr, and M.J. Coon, *J. Biol. Chem.*, 257 (1982) 8472–8480.
15 IUPAC-IUB Joint Commission on Biochemical Nomenclature, *J. Biol. Chem.*, 260 (1985) 14–42.