THE USE OF 4-METHYLUMBELLIFERYL GLYCOSIDES IN BINDING STUDIES WITH THE LECTINS BS I-A_4, BS I-B_4 AND BS II FROM *BANDEIRAEA (GRIFFONIA) SIMPLICIFOLIA*

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1. Introduction

Seeds of *Bandeiraea simplicifolia (Griffonia simplicifolia)* contain 12 lectins and isolectins distributed over the 4 groups BS I–IV [1]. This paper deals with the tetrameric and tetravalent lectins BS I and BS II [2]. BS II is a GlcNAc-specific lectin [3,4]. The BS I group is composed of 5 Gal-binding isolectins A_4, A_3B, A_2B_2, AB_3 and B_4 with degrees of A or B blood-group specificity determined by the ratio of subunit A (GalNAc specific) over subunit B (Gal specific) [5–8]. The degree of α- or β-anomeric binding preference is influenced by the nature of the aglycon; this forms the basis of a reproducible separation of the 5 BS I isolectins by affinity chromatography using aryl β-glycosides as ligand arms [9]. The ligands used here for binding studies in solution are 4-methylumbelliferyl-(MeUmb-) glycosides. Their versatility is apparent from:

(i) The fluorescence of MeUmb α-Galp, MeUmb β-Galp and MeUmb β-GalNAcp that is quenched totally upon binding with BS I-B_4 or almost totally with BS I-A_4;
(ii) The discriminative difference absorption spectra of the MeUmb group when MeUmb α-Galp binds to BS I-A_4 or to BS I-B_4;
(iii) The results of titration of the increase in MeUmb-fluorescence polarisation [10,11] for a series of MeUmb β(GlcNAcp)$_n$ glycosides as ligands for BS II.

Abbreviations: M_r, relative molecular mass; GalNAc, 2-acetamido-2-deoxy-D-galactose; GlcNAc, 2-acetamido-2-deoxy-D-glucose; Me α-Galp, methyl α-D-galactopyranoside; MeUmb α-Galp, 4-methylumbelliferyl α-D-galactopyranoside; MeUmb β-Galp, 4-methylumbelliferyl β-D-galactopyranoside; MeUmb β-GalNAcp, 4-methylumbelliferyl β-2-acetamido-2-deoxy-D-galactopyranoside; MeUmb β-GlcNAcp, 4-methylumbelliferyl β-2-acetamido-2-deoxy-D-glucopyranoside; MeUmb β(GlcNAcp)$_2$, 4-methylumbelliferyl-*N*,*N*′-diacetyl-β-chitobioside; MeUmb β(GlcNAcp)$_3$, 4-methylumbelliferyl-*N*,*N*′,*N*″-triacetyl β-chitotrioside; MeUmb β(GlcNAcp)$_4$, 4-methylumbelliferyl-*N*,*N*′,*N*″,*N*‴-tetraacetyl β-chitotreaoside

2. Materials and methods

BS I-A_4, BS I-B_4 and BS II were obtained as in [9]. BS I-A_4 and BS I-B_4 were dissolved in and dialyzed against 0.05 M 4-(2-hydroxyethyl)-1-piperazine–ethanesulfonic acid (Hepes), 0.15 M NaCl, 1 mM $CaCl_2$ (pH 7.2). For BS II, the same buffer also contained 0.1 mM $MnCl_2$ and 1 mM $MgCl_2$. Concentrations of lectins were determined with ϵ_{280} = 1.41 cm^2/mg for BS I [5] and 1.03 cm^2/mg for BS II [12] and were expressed as binding sites on the basis of M_r = 28 500 for a subunit A and B of BS I [5] and of BS II [12]. The fluorescent ligands were MeUmb β(GlcNAcp)$_n$, with n = 1, 2 or 3 [13,14], MeUmb β(GlcNAcp)$_4$, a gift from Professor Kozo Hamaguchi [15], MeUmb α-Galp, MeUmb β-Galp and MeUmb β-GalNAcp (Koch-Light). These and other carbohydrates (Koch-Light) were dissolved in the appropriate buffer.

Difference absorption spectra were obtained with thermostatted 2 × 0.437 cm tandem mixing cuvettes in a Zeiss PMQII-M4QIII apparatus [16]. Fluorescence measurements were made with an Aminco SPF-500 ratio instrument and spectra were uncorrected. Excitation of MeUmb-glycoside fluorescence was at 325 nm and emission was measured at 373 nm. A ligand solution (0.9–2.9 μM, 604 μl) was contained in a thermostatted 0.4 × 1 × 4.5 cm cuvette. It was titrated by adding a maximal total volume of 130 μl

concentrated protein solution in successive portions with intermediate mixing. All data were corrected for a blank titration without MeUmb glycoside, using the corresponding protein at each temperature. The dilution-corrected titration curves of MeUmb fluorescence and MeUmb-fluorescence polarisation were linearized [17], without approximation concerning the concentration of free protein sites.

3. Results and discussion

Upon binding of GlcNAc to BS II, protein fluorescence with λ_{max} = 329 nm decreases drastically with a shift of λ_{max} to 334 nm. In contrast, protein fluorescence of BS I-A_4 (λ_{max} ~ 325 nm) and especially that of BS I-B_4 (λ_{max} 327 nm) increases upon binding with carbohydrates. With BS I-A_4, this increase is very small and is accompanied by a blue shift as observed with Me α-Galp and with GalNAc. The increase in BS I-B_4 fluoresence, caused by Me α-Galp or by melibiose does not affect the position of the emission maximum.

Many of the above effects, in particular those for BS I-B_4 and especially for BS I-A_4, seemed somewhat small for reliable quantitation of carbohydrate binding. The use of MeUmb glycosides was therefore tested. All effects observed with these ligands (fluorescence quenching, increase in fluorescence polarisation and difference absorption spectra) were abolished by saturating amounts of the corresponding reducing sugar or Me-glycoside.

It was found that the fluorescence of MeUmb α-Galp, MeUmb β-Galp and MeUmb β-GalNAcp is totally quenched upon binding to BS I-B_4 or is quenched to a large extent and in a temperature-dependent way upon binding to BS I-A_4; this allows sensitive evaluation of the association constants K, e.g., at ~13.5°C (fig.1) and at 25°C (table 1). From these data the binding enthalpy, $-\Delta H^\circ$, was estimated to be 15–22 kJ/mol for MeUmb β-GalNAcp or MeUmb α-Galp with BS I-A_4 and 43–49 kJ/mol for MeUmb α-Galp or MeUmb β-Galp with BS I-B_4.

Next to the different carbohydrate specificities of the A and B subunits, the above differences in tryptophane fluorescence upon binding of carbohydrates, in MeUmb-fluorescence quenching and in ΔH° for MeUmb glycoside binding, observed with BS I-A_4 and BS I-B_4, point at different ligand interactions for both homo-isolectins. This can be due in part to the aryl aglycon

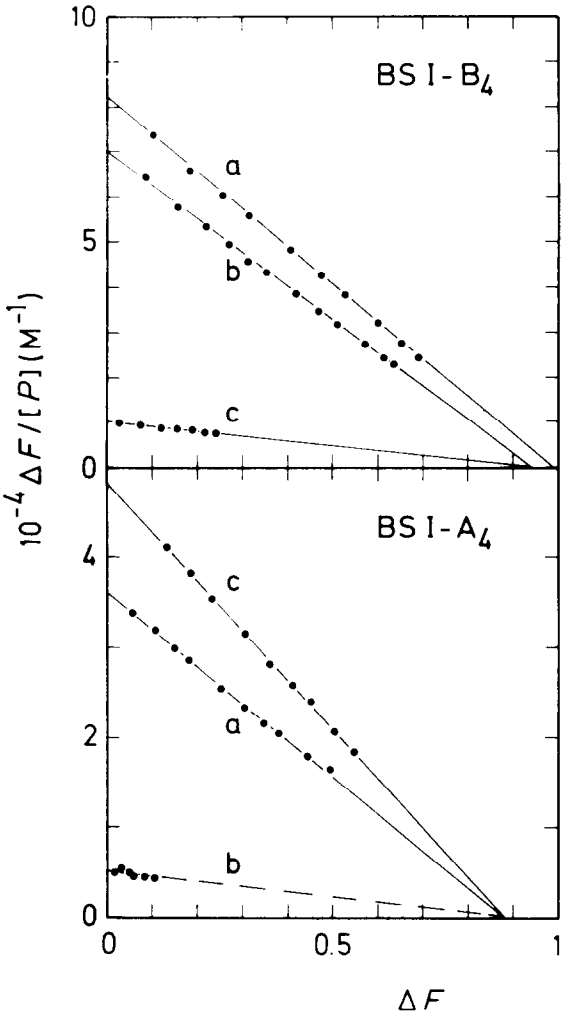

Fig.1. Linearized [15] titration curves of ligand-fluorescence quenching of 0.92 μM MeUmb α-Galp (a), 1.63 μM MeUmb β-Galp (b) and 1.47 μM MeUmb β-GalNAcp (c) by an excess (≤30 μM) of the homo-isolectins BS I-B_4 at 13.1°C (top) and BS I-A_4 at 13.7°C (bottom). ΔF is the relative fluorescence quenching and $[P]$ is the concentration of free sites; both are corrected for dilution. A 1:1 complex was assumed to calculate $[P]$ starting with a value of K corresponding to the slope of a $\Delta F/[P_o]$ *vs* ΔF plot, $[P_o]$ being the concentration of free plus occupied sites. As obtained by iteration, the slope increased to a constant value as illustrated. The values of K (in M^{-1}) and ΔF_∞, at infinite $[P]$, were calculated from the slope and intercept. For BS I-B_4: (8.33 ± 0.05)10^4 and 0.99 ± 0.01 with MeUmb α-Galp; (7.47 ± 0.08)10^4 and 0.94 ± 0.01 with MeUmb β-Galp; (1.04 ± 0.08)10^4 and 0.96 ± 0.07 with MeUmb β-GalNAcp. For BS I-A_4: (5.45 ± 0.07)10^4 and 0.885 ± 0.015 with MeUmb β-GalNAcp; (4.04 ± 0.07)10^4 and 0.89 ± 0.02 with MeUmb α-Galp; ~5.6 × 10^3 and consistent with ΔF_∞ = 0.9 with MeUmb β-Galp.

Table 1
Binding characteristics of MeUmb-glycosides and the two BS I homo-isolectins at 25°C

	ΔF_∞	$10^{-4} \times K$
BS I-A_4		
MeUmb α-Galp	0.77 ± 0.03	2.8 ± 0.1
MeUmb β-GalNAcp	0.79 ± 0.02	4.3 ± 0.1
MeUmb β-Galp	a	a
BS I-B_4		
MeUmb α-Galp	0.99 ± 0.02	4.1 ± 0.1
MeUmb β-GalNAcp	b	b
MeUmb β-Galp	0.98 ± 0.05	3.3 ± 0.1

[a] The data at 13.7°C are consistent with $\Delta F_\infty = 0.90$ and $K = 1.0 \times 10^4$ M^{-1} (fig.1)
[b] At 13.1°C these values are 0.96 and 5.6×10^3 M^{-1} (fig.1)

The data were obtained as in fig.1; see text for $\Delta H°$ values

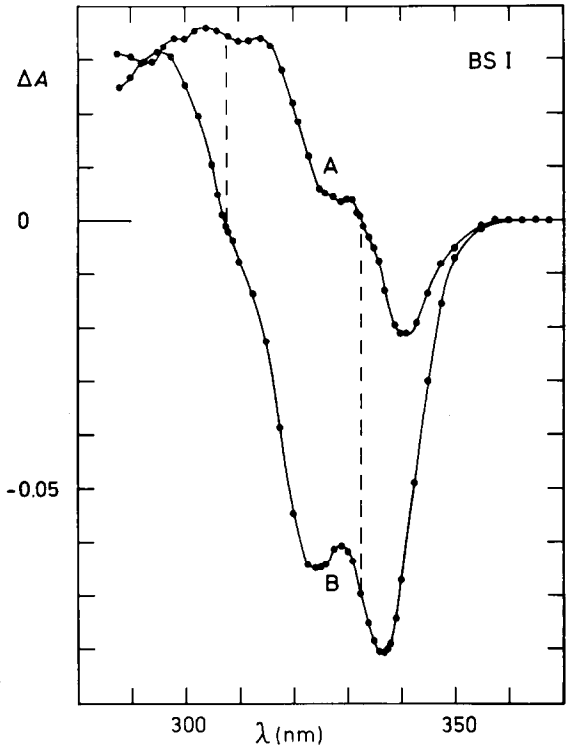

Fig.2. Difference absorption spectra obtained at 13°C with 93 μM MeUmb α-Galp using either 69 μM BS I-A_4 ((A) 45 μM complex) or 91 μM BS I-B_4 ((B) 64 μM complex). When measured at the extreme absorption differences, the MeUmb-difference spectra disappeared in the presence of 40 mM α-Galp. The dashed vertical lines indicate the wavelengths suggested to monitor binding by the A and B subunits in the hetero-isolectins of BS I.

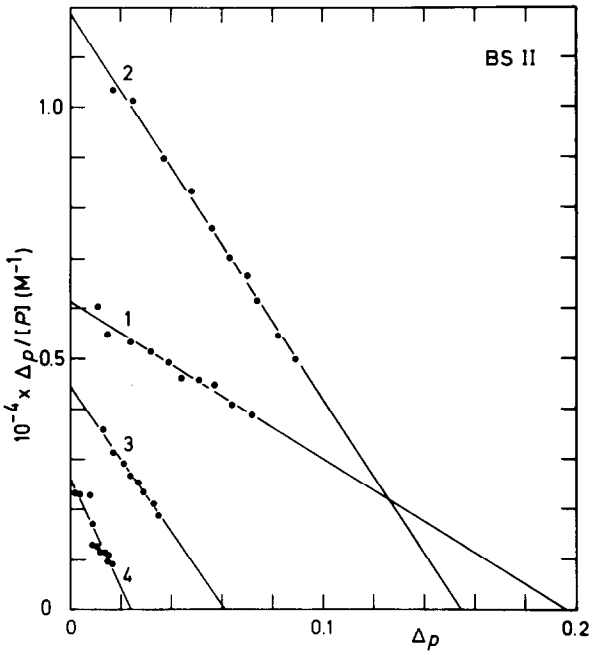

Fig.3. Linearized [15] titration curves of the increase in ligand-fluorescence polarisation of 2.5–2.9 μM MeUmb β(GlcNAcp)$_n$ by an excess (≤20 μM) of BS II at ~13.5°C. n (= 1, 2, 3 or 4 as indicated) is the number of GlcNAc residues in the ligands, Δp is the increase in fluorescence polarisation and $[P]$ was calculated as in fig.1. As checked for the most intense signal changes at the end of the titrations with $n = 1$ and $n = 2$, the initial values of p for free ligand (0.167 for $n = 1$ and 0.223 for $n = 2$) were restored upon addition of a large excess of GlcNAc when corrected for the protein blank; the initial values were $p = 0.250$ for $n = 3$ and $p = 0.251$ for $n = 4$. The values of K (slope) and Δp_∞ (intercept on Δp axis) are gathered in table 2.

since the ratio of K values of the MeUmb glycosides and those of the corresponding p-NO_2Phe glycosides [6,8] are practically identical for BS I-A_4 but are different for BS I-B_4. Indeed, a different surrounding of the MeUmb group is very apparent from the difference absorption spectra when MeUmb α-Galp binds to BS I-A_4 or to BS I-B_4 (fig.2). With BS I-B_4 it arises from a blue shift and shows two minima at 336.5 nm and 325 nm with an isosbestic point at 307 nm which is very similar to the difference absorption spectrum for 4-methylumbelliferyl α-D-mannopyranoside and concanavalin A [16]. With BS I-A_4 the difference absorption spectrum shows a minimum at 340 nm, an isosbestic point at 333 nm and is maximal between 300 and 315 nm. It can be expected that measure-

Table 2
Association constants and increase in MeUmb-fluorescence polarisation for binding of 4 MeUmb β(GlcNAcp)$_n$ glycosides to BS II

No. of GlcNAc	$10^{-4} \times K$ (M^{-1})	Δp_∞
$n = 1$[a]	3.1 ± 0.2	0.196 ± 0.012
$n = 2$[a]	7.7 ± 0.2	0.154 ± 0.004
$n = 3$[b]	7.3 ± 0.3	0.061 ± 0.003
$n = 4$[b]	10.8 ± 1.4	0.024 ± 0.004

[a]14.3°C; [b]13.5°C

The values were obtained by linear regression of the data in fig.3

ments at 333 nm and 307 nm should selectively monitor binding by the B and A subunit in BS I hetero-isolectins; this aspect will be treated elsewhere.

Upon binding to BS II, a positive absorption difference spectrum was observed with MeUmb β-GlcNAcp (not shown); except for its sign, it was similar to the one for the system MeUmb α-Galp · BS I-B_4 (fig.2). When fluorescence of a MeUmb β(GlcNAcp)$_n$ glycoside was measured in the presence of BS II, quenching was very small, if any. This contrasts with the observed fluorescence changes upon binding to wheat germ agglutinin [14,18,19], to lysozyme [13,15,17] and to GlcNAc-directed antibodies [20]. However, binding of MeUmb β(GlcNAcp)$_n$ to BS II could be quantitated by the increase in MeUmb-fluorescence polarisation, Δp, as a measure of the decreased motility of the MeUmb group in a complex. As shown by the data in fig.3 and table 2, K for MeUmb β(GlcNAcp)$_n$ increases and tends to become constant for $n \geqslant 2$. This is consistent with the data for the parent chito-oligosaccharides [3,4]. Moreover, the increase in MeUmb-fluorescence polarisation upon formation of a MeUmb β(GlcNAcp)$_n$ · BS II complex (Δp_∞) decreases as a function of n (table 2). These data, together with the observation that B-D-Gal-(1→4)-D-GlcNAc is an extremely poor ligand, suggest that the carbohydrate-binding site of BS II should accomodate a chitobioside structure through the non-reducing end in a chito-oligosaccharide.

Acknowledgements

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References

[1] Goldstein, I. J. (1981) Intl. Symp. Lectins as Tools in Biology and Medicine, Calcutta, 7–9 January, 1981.
[2] Goldstein, I. J. and Hayes, C. E. (1978) Adv. Carbohyd. Chem. Biochem. 35, 127–340.
[3] Shankar Iyer, P. N., Wilkinson, K. D. and Goldstein, I. J. (1976) Arch. Biochem. Biophys. 177, 330–333.
[4] Ebisu, S., Shankar Iyer, P. N. S. and Goldstein, I. J. (1978) Carbohydr. Res. 61, 129–138.
[5] Hayes, C. E. and Goldstein, I. J. (1974) J. Biol. Chem. 249, 1904–1914.
[6] Wood, C., Kabat, E. A., Murphy, L. A. and Goldstein, I. J. (1979) Arch. Biochem. Biophys. 198, 1–11.
[7] Murphy, L. A. and Goldstein, I. J. (1977) J. Biol. Chem. 252, 4739–4742.
[8] Murphy, L. A. and Goldstein, I. J. (1979) Biochemistry 18, 4999–5005.
[9] Delmotte, F. M. and Goldstein, I. J. (1980) Eur. J. Biochem. 112, 219–223.
[10] Van Landschoot, A., Loontiens, F. G. and De Bruyne, C. K. (1978) Eur. J. Biochem. 83, 277–285.
[11] Kham, M. I., Mathew, M. K., Balaram, P. and Surolia, A. (1980) Biochem. J. 191, 395–400.
[12] Ebisu, S. and Goldstein, I. J. (1978) Methods Enzymol. 50, 350–354.
[13] Delmotte, F. M., Privat, J.-P. and Monsigny, M. (1975) Carbohydr. Res. 40, 353–364.
[14] Van Landschoot, A., Loontiens, F. G., Clegg, R. M., Sharon, N. and De Bruyne, C. K. (1977) Eur. J. Biochem. 79, 275–283.
[15] Yang, Y. and Hamaguchi, K. (1980) J. Biochem. (Tokyo) 88, 829–836.
[16] Loontiens, F. G., Clegg, R. M. and Jovin, T. M. (1977) Biochemistry 16, 159–166.
[17] Yang, Y. and Hamaguchi, K. (1980) J. Biochem. (Tokyo) 87, 1003–1014.
[18] Privat, J. P., Delmotte, F. and Monsigny, M. (1974) FEBS Lett. 46, 229–232.
[19] Erni, B., De Boeck, H., Loontiens, F. G. and Sharon, N. (1980) FEBS Lett. 120, 149–154.
[20] Kieda, C. M. T., Delmotte, F. M. and Monsigny, M. L. P. (1977) Proc. Natl. Acad. Sci. USA 74, 168–172.