PROTON MAGNETIC RESONANCE STUDIES ON WHEAT GERM AGGLUTININ - AMINO SUGAR INTERACTION. EVIDENCE FOR INVOLVEMENT OF A TRYPTOPHAN RESIDUE IN THE BINDING PROCESS

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SUMMARY

The binding of N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and N-acetylneuraminic acid to the lectin wheat germ agglutinin was studied employing proton magnetic resonance titration of the N-acetyl methyl group. A 1:1 association complex fitted the data for the binding of the N-acetyl-D-glucosamine (K_{ass} = 238 M^{-1}) and for N-acetylneuraminic acid (K_{ass} = 560 M^{-1}), but not for N-acetylgalactosamine, which exhibited different binding characteristics. The upfield chemical shift of the bound form of all three substrates implied that the N-acetyl methyl group is located near the face of an aromatic ring. Reaction of wheat germ agglutinin with a tryptophan modifying agent, dimethyl(2-methoxy)-5 nitrobenzyl-sulfonium bromide, eliminated the binding of the monosaccharides, and thus showed the aromatic residue at the binding site to be tryptophan.

INTRODUCTION

The agglutination of cells by wheat germ agglutinin (WGA) may be specifically inhibited by N-acetyl-D-glucosamine (GlcNAc), while N-acetyl-D-galactosamine (GalNAc) is not a hapten inhibitor (1, 2). Several groups have studied the binding of GLcNAc to the lectin either using equilibrium dialysis (3, 4) or fluorescence titration techniques (5, 6). Equilibrium dialysis measurements have shown that the lectin will also bind N-acetyl-neuraminic acid (AcNeu), and the binding of AcNeu is inhibited by the addition of GLcNAc (7). The present investigation directly compared the interaction with the lectin of all three aminosugars, GLcNAc, GalNAc and AcNeu. This paper represents the first reported use of NMR titration techniques to study the binding of saccharides to WGA.

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Abbreviations: WGA, wheat germ agglutinin; MNB-, dimethyl (2-methoxy)-5-nitrobenzyl; DSS, sodium 2,2-dimethyl-2-sialpentane sulfonate.

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MATERIALS AND METHODS

WGA was purified from raw wheat germ using the affinity chromatography method described by Bassett (8). The monosaccharides, GlcNAc, GalNAc and AcNeu, were purchased from the Sigma Chemical Co. WGA was derivatized under gentle aqueous conditions with a modification of Koshland's Reagent, dimethyl(2-methoxy)-5-nitrobenzyl-sulfonium bromide (MNB-sulfonium bromide, Pierce Co.), as described by Horton and Tucker (9).

Hemagglutinating activity of the WGA was measured on a serological scale (0 to ++++) in Cook microtiter plates using freshly washed mouse erythrocytes. A serial 2-fold dilution of either native or modified WGA was added, and the minimum concentration of lectin causing half-maximal (++) agglutination after 1 hr at room temperature was determined. To obtain inhibition titers for GlcNAc, serial dilutions of the hapten were added to either the native or modified lectin prior to adding the cells. Agar double diffusion plates were run with both the native and modified lectin against human erythrocyte solubilized membrane proteins, prepared according to the butanol extraction method of Rega et al (10).

^{1}H NMR measurements were performed at 28°C on a JEOL PS-FT-100 spectrometer (24 K_{Gauss} magnet) operated at 100 MHz in the fast Fourier transform mode. Solutions were prepared in 99.85 ^2H_2O (Stohler Isotopes, Inc.) by dissolving lyophilized WGA in a deuterated buffer. The solution p^{2}H was adjusted to the range 4.5-5.5 p^{2}H in order to achieve the relatively high concentrations of WGA required for the ^{1}H NMR titration studies. Since the experimental technique employed to determine the association constants was dependent on very precise chemical shift measurements, high quality spectra, often of very dilute (10^{-3} M) substrate, had to be collected. Chemical shifts were measured employing two approaches: 1, a direct J.E.C. computer printout, and 2, frequency calibration between the methyl peak of the N-acetyl group and the internal DSS standard (sodium-2)2-dimethyl-2-silapentane sulfonate) on the oscilloscope screen using the "spot" of the instrument with 30 fold horizonatal expansion. Most spectra were collected under water-eliminated Fourier transform conditions, employing 8 K data points, 1 KHz spectral width, 90° pulse, about 12 sec repetition time and 4 sec pulse interval in the T_1 mode. Routinely 25-50 scans were recorded for each data point.

The data treatment method was suggested by the previous work of Dahlquist and Raftery (11) on lysozyme. For a binding process in which the substrate is rapidly exchanging between the bound and unbound environments (as signified by the observation of only a single resonance line in the ^{1}H NMR spectrum), the observed chemical shift, δ_{obs}, is the mole fraction weighted average of the chemical shifts of the free and bound chemical shifts, δ_{free} and δ_{bound}, respectively. Defining $\Delta = \delta_{bound} - \delta_{free}$, and assuming $\Delta >> \delta_{obs}$, it can be shown for a 1:1 complex between monosaccharide, S, and WGA that

$$S_o = [WGA]_o \, \Delta/\delta_{obs} - K_{diss} - [WGA]_o$$

where

$$K_{diss} = [WGA_o\text{-complex}][S_o\text{-complex}]/[complex]$$

Hence a plot of S_o -vs- $1/\delta_{obs}$ provides K_{diss} from the intercept and Δ from the slope.

RESULTS AND DISCUSSION

Table I presents the ^{1}H NMR results and Figure 1 provides a representative data set for GlcNAc. The reproducibility of the chemical shifts was better than 0.002 ppm. The chemical shift of the unbound N-acetyl

Table I

'H NMR DATA FOR LECTIN-MONOSACCHARIDE INTERACTION

Substrate	Substrate Concentration (10^{-3} M)	WGA Concentration* (10^{-4} M)	Number of Points	K_{ass} (M^{-1})	Δ (ppm)
GlcNAc	6-30	6	9	214	1.5
	2-10	2	5	262	1.2
			mean	238	1.35
AcNeu	1.1-5.5	3	5	526	0.41
	2.2-7.7	3	5	656	0.42
	1.1-7.3	2.3	6	498	0.40
			mean	560	0.41
GalNAc	1.2-6	3	6	--	0.03

* Assuming a polypeptide molecular weight of 18,000 daltons.

methyl group was concentration independent at 2,040 ± 0.001 ppm (20 determinations) for all three amino sugars studied. The data on GlcNAc and AcNeu supported the assumption of a 1:1 complex with high linear correlation coefficients. Equilibrium dialysis and fluorescence studies have indicated that the two 18,000 dalton subunits of WGA each possess two binding sites for GlcNAc (4, 12, 13). 'H NMR shows that there is no interaction between these binding sites for GlcNAc. K_{ass} for AcNeu (10^{-3}-10^{-2} M, Table I) is ca. twice that for GlcNAc; both values are very low. The free energy change for binding to WGA at 28°C is ca. 3.3 Kcal/mole for GlcNAc and 3.8 Kcal/mole for AcNeu. Greenaway and LeVine (7) have earlier concluded, on the basis of equilibrium dialysis studies, that over the range 10^{-5}-10^{-6} M WGA has a higher binding affinity for AcNeu than for GlcNAc.

Δ, the upfield chemical shift between bound and free N-methyl

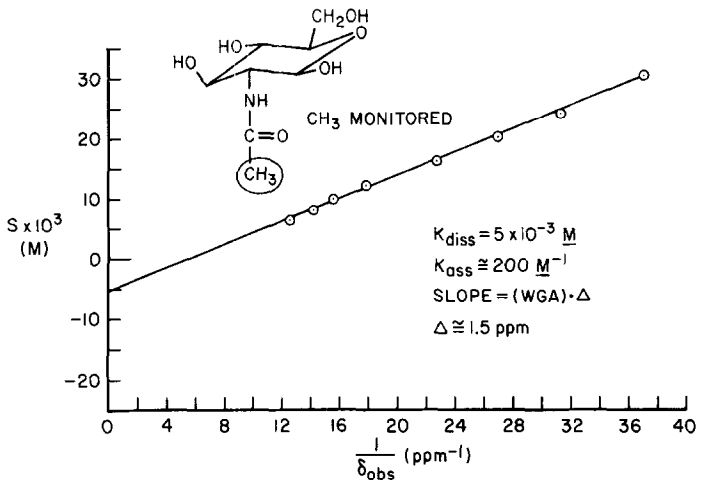

Figure 1. 'H NMR titration of WGA with GlcNAc. WGA concentration was 6.10^{-4} M referred to a polypeptide weight of 18,000 daltons. GlcNAc concentration designated by S.

methyl groups, suggests that in all three bound substrates the methyl group is located near some aromatic residue (14). The large difference in Δ's between AcNeu and GlcNAc implies different orientations of their methyl groups with respect to the aromatic ring.

GalNac provided quantitatively uninterpretable results, but that it was also bound to the lectin was evidenced by the 2.011 ppm chemical shift observed for a GalNAc to WGA molar ratio from 4:1 to 20:1 (compared to 2.040 ppm unbound). Raising the molar ratio to higher values increased δ_{obs} which tended towards δ_{free}. Thus, while binding to WGA is probably taking place, its interaction with the lectin must be different from that of GlcNAc and AcNeu. Allen et al (3) have earlier concluded that the configuration of the 4-OH group must be important in binding, since GalNAc is such a weak inhibitor of WGA compared to GLcNAc.

Modification of the lectin with MNB-sulfonium bromide, specific for tryptophan residues (9), yielded a protein which by spectrophotometric analysis at 410 nm appeared to contain 3.5 ± 0.5 dye molecules per 18,000 dalton subunits. According to the reported amino acid analysis

Table II

AGGLUTINATION OF MOUSE ERYTHROCYTES

Lectin Preparation	Relative Hemagglutinating Activity	Concentration of GlcNAc for 50% Inhibition of Agglutination
WGA (native)	1.00	0.15 mM
WGA- (modified with MNB sulfonium bromide)	0.10	No inhibition observed with >0.10 M

of the purified lectin (4, 8), it is probable that all tryptophan residues had been derivatized by the MNB-sulfonium bromide. Conditions under which δ_{obs} with native WGA clearly indicated binding (molar ratio hapten to WGA of 4:1), with modified WGA gave $\delta_{obs} = \delta_{free}$ for all three substrates. This indicates that the modified lectin could no longer bind the amino sugars to tryptophan, a finding which could be caused by either a blocking of the tryptophan binding site or some conformational change in the protein triggered by the tryptophan modification.

These data support a model, similar to the well documented case of lysozyme, in which the amino sugar GLcNAc is hydrogen bonded via the 3-OH group to a tryptophan N-H on the protein (15). A similarity of AcNeu and GLcNAc binding to the lectin is also suggested by the ^{1}H NMR results.

Table II demonstrates the greatly reduced hemagglutinating activity of the modified lectin compared to the active WGA. Whereas low concentration GLcNAc inhibited activity of native WGA, even 0.1 M GLcNAc did not inhibit the weak agglutinating activity of modified WGA. In contrast to native WGA, the modified WGA did not form a precipitin band with erythrocyte membrane proteins in agar double diffusion (Figure 2).

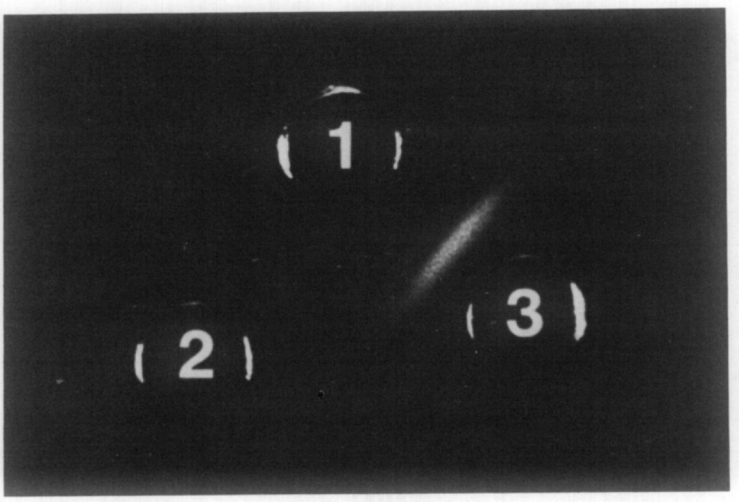

Figure 2. Agar double diffusion plate run at pH 8.4 in barbiturate buffer. Wells (1) solubilized erythrocyge membrane protein, (2) 2 mg/ml WGA-modified with MNB-sulfonium bromide, (3) 2 mg/ml native WGA.

These results further implicate the tryptophan residues of the lectin as binding sites for cell surface carbohydrates.

Very recently, Privat et al (16) have shown that the oxidation of the tryptophan residues of WGA by N-bromosuccinimide led to a decrease in both K_{ass} for the binding of tri-N-acetyl-chitotriose and the relative hemagglutinating activity of the lectin, in excellent agreement with the present work. The binding of AcNeu to WGA reported here is probably of direct physiological significance, since neuraminidase treatment of cells decreases their binding affinity for WGA and also abolishes the lectin-induced agglutinability (1, 17, 18). In addition, we have recently shown that WGA interacts with sialogangliosides incorporated into unilamellar phospholipid vesicles, but does not interact with desialated gangliosides (19).

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REFERENCES

1. Burger, M. M. and Goldberg, A. R. (1967) Proc. Natl. Acad. Sci. (U.S.) 57, 359-366.
2. Redwood, W. R., Jansons, V. K. and Patel, B. C. (1975) Biochim. Biophys. Acta 406, 347-361.
3. Allen, A. K., Neuberger, A. and Sharon, N. (1973) Biochem. J. 131, 155-162.
4. Nagata, Y. and Burger, M. M. (1974) J. Biol. Chem. 249, 3116-3122.
5. Lotan, R. and Sharon, N. (1973) Biochem. Biophys. Res. Commun. 55, 1340-1346.
6. Privat, J. P., Delmotte, F., Mialonier, G., Bouchard, P. and Monsigny, M. (1974) Eur. J. Biochem. 47, 5-14.
7. Greenaway, P. J. and LeVine, D. (1973) Nat. New Biol. 241, 191-192.
8. Bassett, E. W. (1975) Prep. Biochem. 5, 461-477.
9. Horton, H. R. and Tucker, W. P. (1970) J. Biol. Chem. 245, 3397-3401.
10. Rega, A. F., Weed, R. I., Reed, C. F., Berg, G. G. and Rothstein, A. (1967) Biochim. Biophys. Acta 147, 297-312.
11. Dahlquist, F. W. and Raftery, M. A. (1968) Biochemistry 9, 3269-3276.
12. Privat, J. P., Delmotte, F. and Monsigny, M. (1974) FEBS Lett. 46, 224-228.
13. Privat, J. P., Delmotte, F. and Monsigny, M. (1974) FEBS Lett. 46, 229-232.
14. Johnson, C. E. Jr. and Bovey (1958) J. Chem. Phys. 29, 1012-1014.
15. Dahlquist, F. W. and Raftery, M. A. (1958) Biochemistry 9, 3269-3280.
16. Privat, J. P. Lotan, R., Bouchard, P., Sharon, N. and Monsigny, M. (1976) Eur. J. Biochem. 68, 563-572.
17. Adair, W. L. and Kornfeld, S. (1974) J. Biol. Chem. 249, 4696-4704.
18. Cuatrecasas, P. (1973) Biochemistry 12, 1312-1322.
19. Redwood, W. R. and Polefka, T. G. (1976) Biochim. Biophys. Acta 455, 631-643.