Fischer, E. H., Krebs, E. G., & Kent, A. D. (1958) *Biochem. Prep. 6*, 68–73.
Fiske, C. H., & SubbaRow, Y. (1925) *J. Biol. Chem. 66*, 375–400.
Fletterick, R. J., & Madsen, N. B. (1980) *Annu. Rev. Biochem. 49*, 31–61.
Fletterick, R. J., Sygusch, J., Semple, H., & Madsen, N. B. (1976) *J. Biol. Chem. 251*, 6142–6146.
French, D., Knapp, D. W., & Parzur, J. H. (1950) *J. Am. Chem. Soc. 72*, 5150–5152.
Goldemberg, S. H. (1962) *Biochim. Biophys. Acta 56*, 357–359.
Green, A. A., & Cori, G. T. (1943) *J. Biol. Chem. 151*, 21–29.
Hodge, J. E., & Hofreiter, B. T. (1962) *Methods Carbohydr. Chem. 1*, 380–394.
Hu, H. Y., & Gold, A. M. (1975) *Biochemistry 14*, 2224–2230.
Illingworth, B., & Cori, G. T. (1953) *Biochem. Prep. 3*, 1–8.
John, M., Trênel, G., & Dellweg, H. (1969) *J. Chromatogr. 42*, 476–484.
Johnson, L. N., Jenkins, J. A., Wilson, K. J., Stura, E. A., & Zanotti, G. (1980) *J. Mol. Biol. 140*, 565–580.
Kastenschmidt, L. L., Kastenschmidt, J., & Helmreich, E. (1968) *Biochemistry 7*, 3590–3608.
Kasvinsky, P. J., & Madsen, N. B. (1976) *J. Biol. Chem. 251*, 6852–6859.
Kasvinsky, P. J., Madsen, N. B., Fletterick, R. J., & Sygusch, J. (1978) *J. Biol. Chem. 253*, 1290–1296.
Larsson, P. O., & Mosbach, K. (1974) *FEBS Lett. 46*, 119–122.
Martin, G. R., & Ames, B. N. (1961) *J. Biol. Chem. 236*, 1372–1379.
Metzger, B., Helmreich, E.. & Glaser, L. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57*, 994–1001.
Meyer, F., Heilmeyer, L. M. G., Jr., Haschke, R. H., & Fischer, E. H. (1970) *J. Biol. Chem. 245*, 6642–6648.
Nurok, D., & Zlatkis, A. (1978) *Carbohydr. Res. 65*, 265–270.
Philip, G., Gringel, G., & Palm, D. (1979) *Hoppe-Seyler's Z. Physiol. Chem. 360*, 1182.
Schächtele, K. H., Schiltz, E., & Palm, D. (1978) *Eur. J. Biochem. 92*, 427–434.
Seery, V. L., Fischer, E. H., & Teller, D. C. (1967) *Biochemistry 6*, 3315–3327.
Shimomura, S., & Fukui, T. (1980) *Biochemistry 19*, 2287–2294.
Sotiroudis, T. G., Oikonomakos, N. G., & Evangelopoulos, A. E. (1978) *Eur. J. Biochem. 88*, 573–581.
Suter, P., & Rosenbusch, J. P. (1976) *J. Biol. Chem. 251*, 5986–5991.
Tesser, G. I., Fisch, H. U., & Schwyzer, R. (1974) *Helv. Chim. Acta 57*, 1718–1730.
Titani, K., Koide, A., Hermann, J., Ericsson, L. H., Kumar, S., Wade, R. D., Walsh, K. A., Neurath, H., & Fischer, E. H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4762–4766.
Wang, J. H., Shonka, M. L., & Graves, D. J. (1965) *Biochemistry 4*, 2296–2301.
Weber, I. T., Johnson, L. N., Wilson, K. S., Yeates, D. G. R., Wild, D. L., & Jenkins, J. A. (1978) *Nature (London) 274*, 433–437.
Wilchek, M., Oka, T., & Topper, Y. J. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1055–1058.
Wing, R. E., & BeMiller, B. (1972a) *Methods Carbohydr. Chem. 6*, 368–372.
Wing, R. E., & BeMiller, B. (1972b) *Methods Carbohydr. Chem. 6*, 378–384.

Specificity of Isolectins of Wheat Germ Agglutinin for Sialyloligosaccharides: A 360-MHz Proton Nuclear Magnetic Resonance Binding Study†

K. Anne Kronis and Jeremy P. Carver*

ABSTRACT: The binding of three purified sialic acid containing oligosaccharides to two isolectins of wheat germ agglutinin (WGA I and WGA II) has been quantitated by measuring the broadening of a ligand resonance in the proton nuclear magnetic resonance (^{1}H NMR) spectrum at 360 MHz. The ligands, isolated from bovine colostrum by using the procedure of Schneir and Rafelson [Schneir, M. L., & Rafelson, M. E., Jr. (1966) *Biochim. Biophys. Acta 130*, 1–11], were identified by ^{1}H NMR as the α(2,3) and α(2,6) isomers of *N*-acetylneuraminyllactose, as well as the α(2,6) form of *N*,*N*′-diacetylneuraminyllactosamine. The dissociation constants, K_D's, ranged from 0.7 to 10 mM (24 ± 1 °C). Two noteworthy features of WGA specificity emerge from an examination of the observed affinities: (1) both isolectins bind the α(2,3) isomer of *N*-acetylneuraminyllactose with higher affinity than the α(2,6) form and (2) WGA I binds two of the sialyloligosaccharides more tightly than does WGA II.

The use of plant lectins as probes of membrane carbohydrate structure is widespread in many areas of biological research [for reviews, see Nicolson (1974) and Goldstein & Hayes (1978)]. It is generally believed that investigations of lectin–sugar interactions will be essential to our understanding of such processes as differentiation, cancer, and metastasis, since these are theorized to involve "signals" mediated through lectin-like molecules on the external surfaces of cells that bind to specific classes of carbohydrate structures on membranes of other cells. Thus, changes in the array of either the lectin-like membrane proteins or the surface glycoconjugates could alter the fate of the cells involved. Eukaryotic lectins have been observed in many systems and there is mounting

† From the Departments of Medical Genetics and Medical Biophysics, University of Toronto, Toronto, Ontario, Canada M5S 1A8. *Received November 19, 1981.* This research was supported by grants from the Medical Research Council of Canada (MT-3732 and MA-6499) and the National Cancer Institute of Canada.

0006-2960/82/0421-3050$01.25/0

evidence that these endogenous lectins are indeed involved in cell–cell recognition phenomena such as adhesion (Barondes, 1980, 1981).

In recent years it has become increasingly apparent that the physicochemical aspects of lectin–carbohydrate interactions need further investigation, since the specificity of lectins for cell surface oligosaccharides is far more complex than was originally thought on the basis of hapten inhibition studies. Since it is the belief of the authors that only a *molecular* understanding of the carbohydrate specificity of lectins will permit their intelligent utilization in the exploration of the mammalian cell surface, we have undertaken such a study of wheat germ agglutinin (WGA).[1] The importance of gaining insight into the specificity of WGA is paramount as it is one of the more widely used and in some respects the most poorly understood of the plant lectins.

Although it is now generally accepted that WGA is somehow capable of interacting with NeuNAc as well as GlcNAc residues, the specificity for oligosaccharides of biological relevance has remained elusive. It has been suggested that certain of the cell surface NeuNAc residues to which WGA binds occupy the terminal position of Asn-linked complex glycopeptides (Stanley & Carver, 1977; Bhavanandan & Katlic, 1979; Peters et al., 1979). Many representatives of this class of glycopeptide structures contain *N,N'*-diacetylneuraminyllactosamine "antennae", the terminal NeuNAc residues of which are linked either $\alpha(2,3)$ or $\alpha(2,6)$ (Montreuil, 1980). Therefore, we have purified, from bovine colostrum, αNeuNAc(2,3)βGal(1,4)Glc (N3L), αNeuNAc(2,6)βGal(1,4)Glc (N6L), and αNeuNAc(2,6)βGal(1,4)GlcNAc (N6Ln) and have quantitated the affinity of these oligosaccharides for WGA to discern if there is any NeuNAc-linkage specificity in the binding of these antenna analogues by WGA.

Three major isolectins have been identified in WGA preparations (Allen et al., 1973; Rice & Etzler, 1975), and recently, amino acid substitutions have been found at the NeuNAc binding sites of crystals of WGA I and II (Wright, 1981). It is crucial to use homogeneous protein and ligand preparations in binding analyses if a set of data is to be meaningfully interpreted. All previous quantitative binding studies have used mixtures of isolectins. Therefore, we have investigated the affinity of WGA I and WGA II isolectins for the three model sialyloligosaccharides mentioned above.

Experimental Procedures

Analytical Methods. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate ($NaDodSO_4$) and mercaptoethanol was performed in slab gels according to Laemmli (1970) and covered a linear 10–20% gradient in acrylamide. Low molecular weight markers (Bethesda Research Laboratories, Inc.) were included as standards, and the gels were stained with Coomasie Brilliant Blue G (Sigma Chemical Co.). The molecular weight of WGA under reducing conditions was calculated from a plot of migration distance vs. log molecular weight. The method of O'Farrell et al. (1977) was utilized for the nonequilibrium pH gradient electrophoresis (NEPHGE) of WGA and the isolectins. The pH of the focused protein was determined by comparison with p*I* markers as described by Garber & Gold (1982).

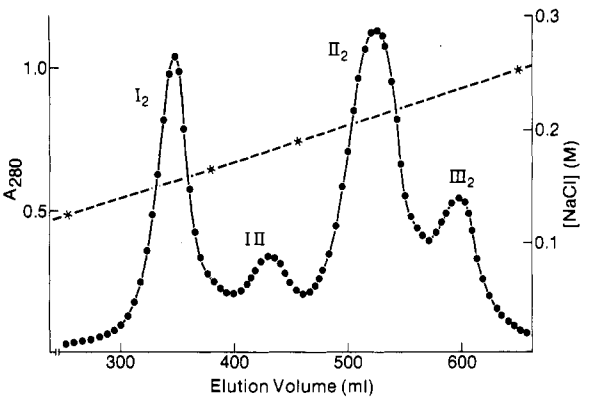

FIGURE 1: CM-Sepharose CL-6B elution profile of WGA isolectins. The fractions were monitored for protein by the absorbance at 280 nm (●). Peaks correspond to WGA dimers: I_2, II_2, and III_2 represent the dimers of WGA I, WGA II, and WGA III, respectively, while I II marks the hybrid dimer formed between isolectins I and II. The salt gradient utilized for the elution of the isolectins was followed by conductivity measurements (*).

Neutral sugars were identified by using the phenol–sulfuric acid test of Dubois et al. (1956) scaled down 5-fold in volume. Results were quantitated with a stock solution prepared from D-mannose, dried in vacuo for 24 h at 70 °C.

WGA Isolation. Unprocessed whole wheat germ was purchased from a local health food store. Crude WGA was purified from the raw wheat germ by ammonium sulfate precipitation (Bassett, 1975) and affinity chromatography on ovomucoid–Sepharose 4B (Marchesi, 1972), as described by Lacelle (1979). A yellow pigment, which copurifies with WGA and is present in commercial WGA samples, was removed by chromatography on CM-Sepharose CL-6B (Lacelle, 1979). WGA was equilibrated in acetate buffer (0.05 M acetate–0.05 M NaCl, pH 4.3) and applied to a CM-Sepharose CL-6B column (1.5 cm × 30 cm) in the same buffer. The column was washed with 2 column volumes of buffer. The mixture of three WGA isolectins was eluted with a high ionic strength buffer (0.05 M acetate–0.45 M NaCl, pH 4.3). The yellow pigment was retained on the column. Aproximately 400 mg of WGA isolectins could be purified from 1 kg of raw wheat germ.

Purification of WGA Isolectins. The three isolectins of WGA have been previously purified by ion-exchange chromatography on SP-Sephadex and QAE-Sephadex (Allen et al., 1973; Rice & Etzler, 1975). In our laboratory, the SP-Sephadex resin did not reproducibly bind the multiple forms of WGA, and the weaker cation exchanger, CM-Sepharose CL-6B, was employed for their separation (Lacelle, 1979). Typically, a mixture of 100–400 mg of WGA isolectins either containing the yellow pigment or after its removal (see above) was equilibrated in buffer (0.05 M acetate–0.05 M NaCl, pH 4.3) at room temperature for several days and was loaded onto the CM-Sepharose CL-6B column as described above. Elution of the isolectins was performed with a linear gradient from 0.05 to 0.45 M NaCl in the acetate buffer (1.2 L in volume) (Figure 1). The peaks were pooled and rerun with this same resin and elution method to remove contaminating isolectins from a given peak. The isolectins were numbered I, II, and III on the basis of their elution order from the cation-exchange resin, in keeping with the original nomenclature of Allen et al. (1973).

The proportions of isolectins in our WGA preparations were 33% WGA I, 49% WGA II, and 18% WGA III. This is

[1] Abbreviations: WGA, wheat germ agglutinin; NeuNAc, *N*-acetyl-D-neuraminic acid; N3L, αNeuNAc(2,3)βGal(1,4)Glc; N6L, αNeuNAc(2,6)βGal(1,4)Glc; N3Ln, αNeuNAc(2,3)βGal(1,4)GlcNAc; N6Ln, αNeuNAc(2,6)βGal(1,4)GlcNAc; K_D, dissociation constant; ΔG, Gibbs free energy of binding; CHO, Chinese hamster ovary; BHK, baby hamster kidney; NEPHGE, nonequilibrium pH gradient electrophoresis; CM, carboxymethyl; SP, sulfopropyl; QAE, [diethyl(2-hydroxypropyl)-aminoJethyl; ^{1}H NMR, proton nuclear magnetic resonance.

roughly equal to the ratio found by Rice & Etzler (1975), namely, 35% WGA I, 55% WGA II, and 10% WGA III. The covalent dimer WGA IIb, reported in that work to represent 5% of the total WGA, has not been separated from the WGA II preparations in our laboratory.

Characterization of WGA and Isolectins. WGA free of the yellow pigment (see above) was characterized by amino acid composition (Lacelle, 1979), by the aromatic region of the ^1H NMR spectra, by molecular weight determination on NaDodSO$_4$–polyacrylamide gel electrophoresis, and by NEPHGE. The amino acid compositions of the isolectins were similar to those that have been reported (Allen et al., 1973; Rice & Etzler, 1975). The most notable difference in the isolectins is the presence of two histidine residues per monomer in both WGA II and WGA III while WGA I has none (Rice & Etzler, 1975; Lacelle, 1979). The WGA isolectins purified in our laboratory exhibited protein NMR spectra that were consistent with the reported amino acid composition. WGA II possesses a signal at 8.7 ppm at pD 5.0 (assignable to the C2 protons of His residues) that is absent in WGA I under similar conditions (Lacelle, 1979). The occurrence of His residues is one of the few known criteria for distinguishing WGA II from WGA I. WGA ran as a single band on reducing NaDodSO$_4$–polyacrylamide gel electrophoresis when stained with Coomassie Blue (data not shown). On the basis of its mobility relative to protein standards, the molecular weight of WGA monomers was calculated to be 17 000 ± 1000, in agreement with previous reports (Nagata & Burger, 1974; Rice & Etzler, 1974). WGA is a basic protein (Rice & Etzler, 1975) and was found to run as such on NEPHGE (data not shown). WGA I and WGA II focused in the pH range 8.4–8.9, in agreement with the results of Rice & Etzler (1975).

Purification of Three Sialyloligosaccharides. The NeuNAc-containing trisaccharides were purified by a slight modification of the procedure of Schneir & Rafelson (1966). Bovine colostrum was kindly provided by Dr. Carl Grant, The Hospital for Sick Children, Toronto, Canada. The colostrum (230 g) was dialyzed against 8.8 L of distilled water at 4 °C. The dialyzate was applied to an AG1-X2 column (200–400 mesh, acetate form, 5 cm × 100 cm). The column was washed with 7.3 L of distilled water, and the acidic sugars were eluted with a concave pyridyl–acetate gradient as in the original procedure. Fractions (20 mL) were monitored for neutral sugar by using the phenol–sulfuric acid test (see above). Every tenth sugar-containing fraction was lyophilized, dissolved in distilled water, and relyophilized until the traces of pyridine had been removed. The lyophilized fractions were exchanged 2 or more times in deuterium oxide (see NMR Sample Preparation) and were analyzed by 360-MHz ^1H NMR to determine the purity of the sample and to identify the component sugar(s), thus monitoring the fractionation of the sialyloligosaccharides on the ion-exchange resin.

NMR Characterization of Sialyloligosaccharides. The procedure of Schneir & Rafelson (1966) has been used in several laboratories for the purification of sialyloligosaccharides from bovine colostrum. Although three main compounds were separated in the original work, only the $\alpha(2,3)$ and $\alpha(2,6)$ isomers of *N*-acetylneuraminyllactose were identified at that time. Recently, the third NeuNAc-containing oligosaccharide, which eluted prior to N6L, has been shown to be N6Ln (Veh et al., 1981). We also have purified this trisaccharide from bovine colostrum and, in agreement with Veh et al. (1981), have found it to be the main contaminating species present in commercial preparations of *N*-acetylneuraminyllactose (Sigma Chemical Co., type I).

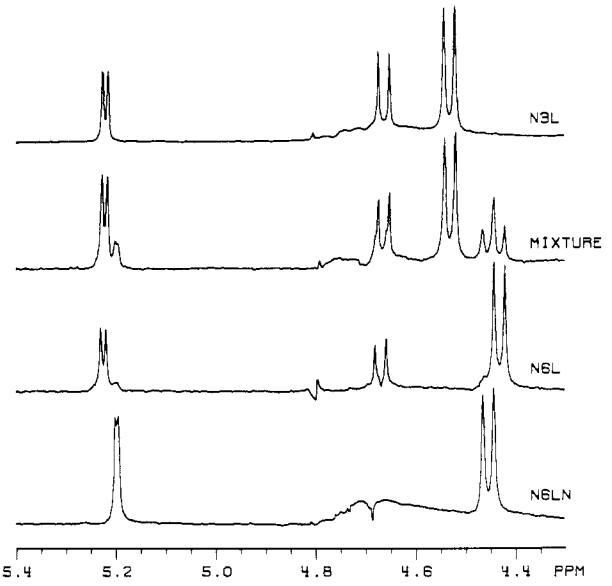

FIGURE 2: Region of anomeric resonances of the 360-MHz ^1H NMR spectra of N3L, the mixture of ligands prior to their separation, N6L, and N6Ln. The samples were run at 24 ± 1 °C in D_2O. The fractionation of the sialyloligosaccharides was monitored by the chemical shift of the β-Gal anomeric resonance as described under Experimental Procedures: N6L, 4.430 ppm; N6Ln, 4.454 ppm; N3L, 4.530 ppm. The remaining peaks in this region have been assigned to the C1–H of the reducing Glc(NAc): N3L, 4.662 (β) and 5.220 ppm (α); N6L, 4.668 (β) and 5.222 ppm (α); N6Ln, the β resonance is obscured by the suppression of the residual HDO peak and the α resonance is at 5.197 ppm.

The detailed structural characterization by 360-MHz ^1H NMR of these three major sialyloligosaccharides will be reported elsewhere (K. A. Kronis and J. P. Carver, unpublished results). However, the anomeric regions of the ^1H NMR spectra for a mixture of the three sialyloligosaccharides as well as the three purified sugars are depicted in Figure 2. Examination of a few informative regions of the NMR spectra revealed that the three main trisaccharides could be conveniently monitored throughout their purification [see Dorland et al. (1978) for N3L and N6L]. For instance, the chemical shift of the Gal C1–H doublet is distinct for all three sugars: the resonance of N6L (4.430 ppm) is shifted +0.100 ppm when the Gal residue is 3 substituted as in N3L, and it is also sensitive to the nature of the sugar to which it is linked (+0.024 ppm in N6Ln compared with N6L, Figure 2). Also, the doublet of doublets corresponding to the equatorial C3–H of αNeuNAc has a chemical shift that is distinct for the three sugars.

The purity of the samples used in the titrations was determined by examining the spectra. No contamination of N3L or N6Ln was found. The N6L samples, however, were 8% contaminated by N6Ln (see Figure 2).

NMR Sample Preparation. Purified WGA isolectins were exchanged 3 or more times in deuterium oxide (D_2O) as follows. Lyophilized protein samples were equilibrated for several hours at room temperature in 99.7% D_2O (Merck Sharp & Dohme). The samples were then lyophilized over P_2O_5. The concentration of WGA I or WGA II samples was calculated based on the absorbance at 277 nm and the reported extinction coefficients at this wavelength, namely, 1.70 and 1.60 cm^2/mg for WGA I and WGA II, respectively (Rice & Etzler, 1974).

Sugar samples were exchanged 2 or more times in 99.7% D_2O as described for WGA isolectins. The concentration of

Table I: Summary of Binding Experiments between WGA I and WGA II and Three Sialyloligosaccharides at 24 ± 1 °C

isolectin (P)	$[P]_T$ (μM)	ligand (A)	$[A]_T$ range (mM)	pD	no. of points	K_D[a] (mM)	correln coeff[b]
WGA I	43	N3L	0.8–4.3	6.14	16	0.74	0.949
WGA I	85	N3L	0.8–10.8	6.30	9	0.87	0.940
WGA I	109	N6L	0.9–6.0	6.13	8	5.3	0.978
WGA I	91	N6Ln	1.5–9.3	6.30	11	9.5	0.885
WGA II	85	N3L	1.5–9.0	5.90	11	3.6	0.955
WGA II	93	N6L	0.9–10.4	6.13	10	10.8	0.987
WGA II	85	N6Ln	0.9–10.4	6.13	11	10.6	0.925

[a] K_D values were obtained from the y intercept of the least-squares fits of plots of $[A]_T$ vs. $\Delta\Delta\nu^{-1}$ (see Figure 3A,B for WGA I and Figure 3C for WGA II) as described in the text. [b] The correlation coefficient was calculated as described in Steel & Torrie (1960).

sugar in a sample was calculated based on the dry weight after lyophilization over P_2O_5 and the appropriate molecular weight (633.6 for *N*-acetylneuraminyllactose and 674.6 for *N,N'*-diacetylneuraminyllactosamine). The concentration was sometimes verified by assaying for neutral sugar or for NeuNAc and was always within 5% of the value calculated by weight.

Deuterated buffer (0.1 M NaP_i–0.15 M NaCl in D_2O), used in the binding titrations, was prepared in the following manner. The mono- and dibasic salts of sodium phosphate as well as NaCl were exchanged several times in 99.7% D_2O as described for lyophilized isolectin samples. Deuterium oxide (99.96%, Merck Sharp & Dohme) was added to the appropriate amounts of exchanged salts. The pD was calculated by adding 0.4 to the reading of a conventional pH meter equipped with a glass combination electrode (Glasoe & Long, 1960). The pD of different buffer preparations ranged from 5.9 to 6.3 (Table I).

Instrumental Conditions. Proton magnetic resonance spectroscopy was performed at the Toronto Biomedical NMR Centre, University of Toronto, on a 360-MHz Nicolet spectrometer. Spectra were collected in the Fourier transform mode at the ambient probe temperature of 24 ± 1 °C. The spectral conditions were 5-kHz sweep width, 5-s pulse delay, 16 K data points, and 16–512 scans, the latter depending on the ligand concentration. The broad signal in the region 4.7–4.8 ppm arises from the partially supressed resonance from residual HDO. Chemical shifts were measured relative to internal acetone (2.225 ppm relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate). The acetone, present at a concentration of 4–8 mM, also served as a field homogeneity standard. Sample volumes ranged from 400 to 800 μL and were analyzed in 5-mm NMR tubes (catalogue no. 528PP, Wilmad Glass Co. Inc.).

Binding Experiments. Six titrations were performed between WGA I or WGA II and each of three sialyloligosaccharides. In all cases, a stock protein solution was made by dissolving D_2O-exchanged isolectin in deuterated buffer and centrifuging at 15000*g* for 1 min. The OD_{277} of diluted supernatant aliquots was used to calculate the isolectin concentration. The stock was split into two 1-mL portions, to one of which was added dry, lyophilized ligand. The different sugar concentrations required for the titration were obtained by mixing various amounts of the two samples. Thus, the protein concentration was fixed during a titration, usually near 100 μM (see Results). In the case of WGA I binding to N3L, separate titrations were conducted at three different protein concentrations, namely, 43, 85, and 192 μM, to verify that there were no concentration-dependent changes in K_D values.

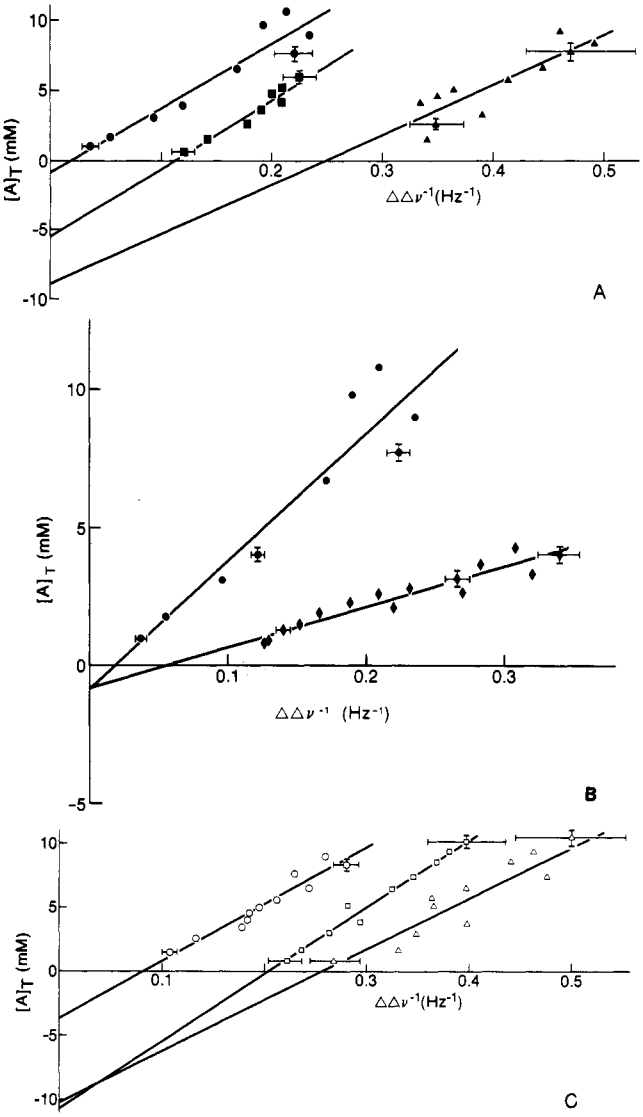

FIGURE 3: Binding of N3L, N6L, and N6Ln to WGA I and WGA II. The total ligand concentration, $[A]_T$ (millimolar), is plotted as a function of the reciprocal of the net change in line width, $\Delta\Delta\nu^{-1}$ (Hz^{-1}). Straight lines were determined by using a least-squares fit program. The y intercept reflects the K_D value. (A) For comparative purposes, the titrations conducted at similar WGA I concentrations have been plotted on the same graph: [WGA I] of 85 μM for N3L (●), 109 μM for N6L (■), and 91 μM for N6Ln (▲). (B) The titrations involving N3L and two concentrations of WGA I, reported in Table I, are plotted to show the effect of protein concentration on the slope but not on the y intercept and hence not on the K_D value: [WGA I] of 85 (●) and 43 μM (♦). (C) The binding of N3L, N6L, and N6Ln to WGA II: the titrations were performed at WGA II concentrations of 85 μM for N3L (○), 93 μM for N6L (□), and 85 μM for N6Ln (△).

These experiments afforded similar K_D values (in the range 0.7–1.1 mM), indicating that the assumption of simple bimolecular association is valid and that the protein sample viscosity was not a factor in the broadening of the ligand resonances (Figure 3B and Table I).

Analysis of Binding Data. In the binding equilibrium between a small ligand A and a macromolecule P, a resonance of a particular nucleus of A may shift and/or broaden due to chemical exchange between the free and bound environments. These spectral changes are related to X_B, the fraction of ligand bound ($[A]_B/[A]_T$), by the equations derived by Swift & Connick (1962). These two types of spectral perturbations have been used successfully in characterizing the binding of small molecules to proteins in general (Dahlquist & Raftery,

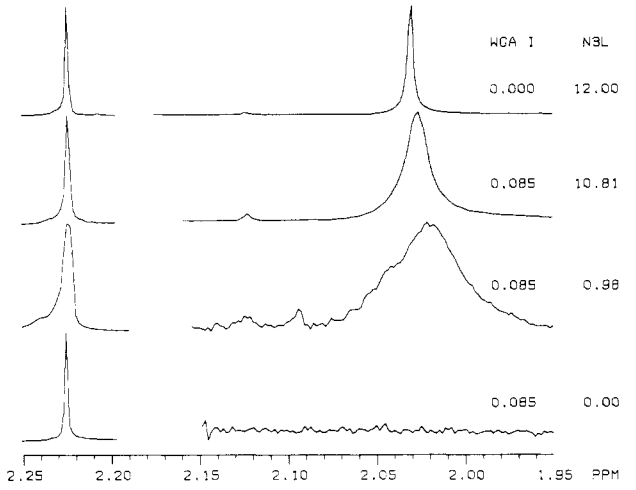

FIGURE 4: WGA I induced broadening of the *N*-acetyl resonance of N3L. The concentrations (millimolar) of WGA I and N3L for each spectrum are indicated under the appropriate heading: the upper spectrum is of the free sugar; the lower spectrum is of pure isolectin; the middle two spectra represent different fractions of bound ligand. The spectra include the acetone resonance (2.225 ppm) and the ligand *N*-acetyl resonance (2.030 ppm for the free sugar and to higher field upon binding). The broadening of the *N*-acetyl resonance was measured to generate binding data as described in the text.

1968; Lanir & Navon, 1971) and to WGA in particular (Jordan et al., 1977, 1981; Grivet et al., 1978; Lacelle, 1979). In the ^{1}H NMR experiments described below, intermediate exchange conditions prevailed at 24 ± 1 °C for all six titrations (K. A. Kronis and J. P. Carver, unpublished results). Although the NeuNAc methyl resonance experienced an upfield shift upon binding, the magnitude of the change was too small in several of the titrations to be useful in accurate K_D determinations. The change in the line width of the methyl signal of NeuNAc was therefore measured instead (see Figure 4).

In the presence of a macromolecule, the observed line width at half-height of a ligand resonance, $\Delta\nu_{obsd}$, is given by

$$\Delta\nu_{obsd} = \Delta\Delta\nu + \Delta\nu_F + I \quad (1)$$

where $\Delta\Delta\nu$ is the net change in line width due to interaction with the protein, $\Delta\nu_F$ is the free line width, and I is the field inhomogeneity. I was taken to be the line width of internal acetone in each spectrum and ranged from 0.3 to 0.8 Hz. The net change in line width, $\Delta\Delta\nu$ (hertz), can be related to the apparent bound line width, $1/(\pi T_{2Bapp})$ (hertz), by

$$\Delta\Delta\nu = X_B/(\pi T_{2Bapp}) \quad (2)$$

Swift & Connick (1962) derived an expression that relates $1/(T_{2Bapp})$ to various parameters that characterize the particular binding process under study. For the purpose of this study, eq 2 is sufficient to allow K_D to be determined. A more detailed description of these parameters will be reported elsewhere (K. A. Kronis and J. P. Carver, unpublished results).

The dissociation constant, K_D (molar), which quantitates the affinity of A for P, is given by

$$K_D = (n[P]_T - [A]_B)([A]_T - [A]_B)/[A]_B \quad (3)$$

where $[P]_T$ is the total monomer concentration of P and n is the number of binding sites for A per monomer. If $[A]_B$ is small compared with $[A]_T$ ($X_B \ll 1$), then eq 3 simplifies to

$$K_D = n[P]_T/X_B - [A]_T \quad (4)$$

Solving eq 2 for X_B and substituting X_B into eq 4 gives

$$[A]_T = n[P]_T/(\pi T_{2Bapp}\Delta\Delta\nu) - K_D \quad (5)$$

Thus a set of $\Delta\Delta\nu$ values for the various ligand concentrations throughout a titration may be plotted as $[A]_T$ vs. $\Delta\Delta\nu^{-1}$, generating a straight line with a y intercept of $-K_D$ and a slope of $n[P]_T/(\pi T_{2Bapp})$. The number of WGA binding sites for NeuNAc-containing oligosaccharides in solution has not been measured. Since n is not a factor in the y intercept, no assumptions were necessary about the value of n in the determination of K_D values.

Table II: Comparison of the Gibbs Free Energy of Binding for the Six Titrations[a]

ligand	ΔG[b] (WGA I) (kcal/mol)	ΔG (WGA II) (kcal/mol)	$\Delta\Delta G$[d] (I-II) (kcal/mol)
N3L	−4.2[c]	−3.3	−0.9
N6L	−3.1	−2.7	−0.4
N6Ln	−2.7	−2.7	0
$\Delta\Delta G$(3-6)[d] (kcal/mol)	−1.1	−0.6	
$\Delta\Delta G$(L-Ln)[d] (kcal/mol)	−0.4	0	

[a] The Gibbs free energy of binding, ΔG, was calculated by using $\Delta G = RT \ln K_D$, where R = 1.99 kcal/(mol·K) and T = temperature (K). [b] A rigorous statistical analysis of the errors in the intercepts has not been included in this work, and thus no errors are quoted on these ΔG or $\Delta\Delta G$ values. From repeated experiments, it was found that the errors in ΔG determination for the titrations that are compared were roughly ±0.1 kcal/mol. Thus, the errors on the $\Delta\Delta G$ values can be taken as ±0.2 kcal/mol or less. [c] This value represents the average of four determinations, two of which are reported in Table I. [d] $\Delta\Delta G$(I-II) = ΔG(WGA I) − ΔG(WGA II). $\Delta\Delta G$(3-6) = ΔG(N3L) − ΔG(N6L). $\Delta\Delta G$(L-Ln) = ΔG(N6L) − ΔG(N6Ln).

The line widths and their errors were measured in this work by the Lorentzian line-fitting routine of the Nicolet 1180 computer. The errors in $\Delta\Delta\nu$ values (shown in Figure 3 as errors in $\Delta\Delta\nu^{-1}$) were computed as the sum of the two individual errors. The data were analyzed by linear regression analysis, and the y intercepts represent those of the least-squares fit. A rigorous statistical analysis of the errors in the intercepts has not been included in this work, and thus no errors have been quoted on the K_D values or their corresponding ΔG values. From four determinations involving WGA I and N3L, it was found that the ranges in K_D values represented ΔG ranges of 0.2 kcal/mol. Therefore it has been concluded that the errors in ΔG's are roughly ±0.1 kcal/mol and the errors in the $\Delta\Delta G$ values can be taken as ±0.2 kcal/mol or less.

Results

Specificity of WGA Isolectins. So that the specificity of WGA isolectins for the linkage of NeuNAc residues could be determined, the affinities of N3L and N6L for a particular isolectin have been compared. The K_D of WGA I for N3L is less than that for N6L, demonstrating that this isolectin has a higher affinity for the α(2,3) isomer (Table I). Graphically, this is represented in Figure 3A where the less negative y intercept reflects the smaller K_D. Similarly, an examination of WGA II binding to these two sugars indicates that N3L is bound more tightly than N6L, again a preference for the α(2,3) linkage (Table I, Figure 3C).

In order to examine the thermodynamic and structural bases for the differing affinities, it is useful to express them as the Gibbs free energies of binding. The ΔG's for the six binding experiments, as well as differences in ΔG values ($\Delta\Delta G$), are listed in Table II. It should be noted that the more negative the ΔG value, the more free energy of binding and, hence, the stronger the interaction. The K_D of 0.8 mM, which characterizes the affinity of WGA I for N3L, represents a ΔG of −4.2 kcal/mol. The weaker interaction between WGA I and N6L

yields a ΔG of −3.1 kcal/mol. The difference in these two free energies, $\Delta\Delta G$, reflecting the preference of WGA I for N3L over N6L, is −1.1 kcal/mol. In an analogous manner, it has been determined that WGA II binds the $\alpha(2,3)$ isomer of *N*-acetylneuraminyllactose with more negative free energy than the $\alpha(2,6)$ form. The magnitude of the difference in free energies is 0.6 kcal/mol.

The effect of substituting a GlcNAc for the Glc at the reducing end of the trisaccharides can be measured by comparing the affinity of one isolectin for N6L with that for N6Ln. The data for WGA I indicated that N6L is bound more tightly than N6Ln (Table I) with a $\Delta\Delta G$ of −0.4 kcal/mol (Table II and Figure 3A). However, the K_D's for these two sugars binding to WGA II are essentially the same. This indicates that the relatively weak affinities of WGA II for N6L and N6Ln are indistinguishable by using this NMR binding technique (Figure 3C). Unfortunately, a comparison of this potential GlcNAc effect could not be made in the $\alpha(2,3)$ series due to the unavailability of this ligand at present.

Comparison of WGA I and WGA II. The effects of amino acid substitutions in the NeuNAc binding sites of WGA I and WGA II are revealed by the differences in their affinities for the same ligand. The $\alpha(2,3)$ form of *N*-acetylneuraminyllactose is bound more tightly by WGA I than by WGA II (Tables I and II). The difference in the K_D's represents a $\Delta\Delta G$ of −0.9 kcal/mol. In the case of N6L, the $\Delta\Delta G$ was calculated to be −0.4 kcal/mol.

Discussion

WGA Isolectins Bind NeuNAc in α(2,3) Linkage with Higher Affinity. The results of the NMR titrations with the two linkage isomers of *N*-acetylneuraminyllactose reveal that both WGA I and WGA II bind the $\alpha(2,3)$ form more tightly than the $\alpha(2,6)$ isomer. Although the binding of NeuNAc to WGA has been previously reported (Jordan et al., 1977, 1981; Wright, 1980a,b), the above data represent the first demonstration of WGA specificity for the *linkage* of the terminal NeuNAc residue of sialyloligosaccharides. For WGA I, the observed difference in the free energy of binding between N3L and N6L is −1.1 kcal/mol. This may represent an additional enthalpic interaction with the binding site or a difference in the entropy of binding for the two ligands. Experiments to clarify this point are under way in our laboratory.

Although the solution conformations of N3L and N6L are not known, it is reasonable to assume that their three dimensional structures will be quite different. The Gal residue of the lactose moiety is substituted by NeuNAc at opposite sides of the pyranose ring in N3L and N6L (at C3 and C6, respectively). In addition, glycosidic linkages to the C6 position of a sugar, as occurs in N6L, are unique in that there exists an extra angle of rotation between the sugars—about the C6–C5 bond. A combination of these factors presumably results in distinct solution conformations for these trisaccharides. Since the primary interaction of WGA for N3L is with the NeuNAc residue (Wright, 1980b), the difference in affinity for the two linkage isomers must arise from the altered orientation of the lactose moiety relative to the protein surface. Further evidence that the lactose group affects the interaction with WGA is provided by the observation that the affinity for WGA I is decreased when the reducing terminal glucose is replaced by an *N*-acetylglucosamine residue (i.e., from N6L to N6Ln). Elucidation of the solution conformations of these sialyloligosaccharides and of larger glycopeptides containing the linkage isomers of NeuNAc is now in progress.

The NeuNAc-containing sequences present in the Asn-linked glycopeptides of cell surface glycoproteins are αNeuNAc(2,3 or 2,6)βGal(1,4)βGlcNAc, i.e., N3Ln or N6Ln (Montreuil, 1980). Because these structures have been shown to be involved in high-affinity binding of WGA to CHO cells (Stanley & Carver, 1977), it was surprising to find that N6Ln was bound less tightly by WGA I than N6L. However, the αNeuNAc(2,3)βGal(1,4)βGlcNAc sequence may yet prove to have a higher affinity than N3L for WGA I. As a result of their different three-dimensional shapes, the *N*-acetyllactosamine moiety may be oriented more favorably in relation to the protein surface in the $\alpha(2,3)$ series than it was in N6Ln.

Several lines of evidence suggest that the observed $\Delta\Delta G$ between N6L and N3L (Table II) may reflect the specificity of WGA binding to the cell surface. Structural studies on cell-derived material from WGA-resistant B16 mouse melanoma cells (Finne et al., 1980) were interpreted as showing a reduction in NeuNAc residues that had been linked to the 3 position of penultimate Gal residues with a concomitant increase in fucose in $\alpha(1,3)$ linkage to GlcNAc. No decrease in αNeuNAc(2,6)Gal sequences was observed, and the O-linked structures were unaltered. On the assumption that WGA resistance results from decreased binding, the decrease in the cytotoxic effect of WGA on this clone may be attributed to a specific loss in αNeuNAc(2,3)Gal sequences at the terminal position of complex glycopeptides. More recently, the phenotype of this mutant clone has been shown to arise from an increased fucosyltransferase activity (Finne et al., 1982).

Another possible indication that WGA is specific for cell surface $\alpha(2,3)$-linked NeuNAc is the observation that three genetically distinct Wga^R CHO cell lines all show a considerable reduction in membrane sialic acid compared to parental cells (Stanley et al., 1980). It has previously been reported that in wild-type CHO cells greater than 80% of the NeuNAc in cell surface glycopeptides is linked $\alpha(2,3)$ (Briles et al., 1978). Thus, the WGA-resistant phenotypes may have arisen in these mutants through a specific loss of $\alpha(2,3)$-linked NeuNAc from different classes of glycopeptide structures at the surface.

WGA I Binds Two Sialyloligosaccharides More Tightly Than Does WGA II. The results of the NMR titrations that have been performed in this study, summarized in Tables I and II, reveal that WGA I binds both N3L and N6L with more negative free energy than does WGA II. This represents the first report in which the binding of different isolectins of WGA to individual ligands has been quantitated by using the same binding technique.

The most prominent difference in the amino acid compositions of WGA isolectins is the absence of histidine residues in WGA I in contrast to two histidines per monomer for each of the other isolectins (Rice & Etzler, 1975). One of these His residues (residue 64) has been located in the NeuNAc binding site of WGA II crystals (Wright, 1980a,b, 1981). The carbonyl of the NeuNAc (or GlcNAc) acetamido group in this subsite is in potential hydrogen bond distance to the backbone N–H of His-64 in WGA II (Wright, 1980a,b). The glycerol side chain of NeuNAc (C7, C8, C9) may interact with His-64 as well (Wright, 1980a). The identity of residue 64 of WGA I is not certain but is thought to be that of a Phe or a Tyr residue (Wright, 1981). Since the conformations of the peptide backbone of these two isolectins are believed to be very similar (Wright, 1980a), we can presume that the differences in the side chains at residue 64 might account for the binding of N3L and N6L to WGA I with higher affinity than to WGA II.

Aromatic Residues at Sugar Binding Sites. The NeuNAc methyl resonance of N3L and N6L was shifted to higher field

when binding to WGA I or WGA II occurred (see Figure 4 for WGA I and N3L). A shift such as this is usually the result of the proximity of a ligand group to either a paramagnetic center or a ring current in the binding site. Since WGA binds no metals and there is no other evidence in the NMR spectrum for the presence of paramagnetic metal ions, it has been concluded that an aromatic amino acid side chain provides the source of the ring current shifts. The upfield direction of the shift suggests that the NeuNAc methyl group of both N3L and N6L is oriented in the isolectin binding sites in a similar fashion over the face of an aromatic amino acid. Wright (1980b) found the methyl group of N3L to be in van der Waals' contact with the side chain of residue 71, which she has tentatively assigned as a tyrosine. Wright (1980b) has also shown superimposition of the *N*-acetyl groups of the terminal NeuNAc of N3L and the "nonreducing" terminal GlcNAc of βGlcNAc(1,4)GlcNAc in their complexes with WGA II. The solution NMR studies of Lacelle (1979) on GlcNAc-containing oligomers binding to WGA I indicated the presence of a Tyr residue at the site of interaction with the nonreducing terminal GlcNAc residue. Thus, the orientation of the *N*-acetyl groups of bound NeuNAc and GlcNAc, observed in the NMR solution studies, is in agreement with that observed in the crystal lattice.

Binding of WGA to Cells. The affinities that have been reported here for the interactions of the isolectins of WGA with NeuNAc-containing trisaccharides are orders of magnitude weaker than those for WGA binding to mammalian cells (Adair & Kornfeld, 1974; Monsigny et al., 1980; P. Stanley and J. P. Carver, unpublished observation). There are several lines of argument that can be raised in order to explain this fact, which are based solely on the exponential relationship between K_D values and the corresponding ΔG's. The simultaneous binding of one WGA monomer to two carbohydrate structures that are attached would result in a tremendous increase in affinity of WGA for that entire structure due to the additivity of the component ΔG values. For instance, the binding of one WGA dimer to two N3L-type moieties at the cell surface would result in a heightened affinity over that which has been reported in this work for free N3L groups. The ΔG for WGA I binding to two N3L groups would be twice −4.2 kcal/mol or −8.4 kcal/mol. Whereas the K_D for one N3L was 8×10^{-4} M, the affinity for two attached N3L's would become 6×10^{-7} M. K_D values in this range have been observed for the binding of WGA to cells: 1×10^{-6} M for BHK cells (Monsigny et al., 1980); 1.3×10^{-5} M and 1×10^{-6} M for the low- and high-affinity binding sites of human erythrocytes (Adair & Kornfeld, 1974); 5×10^{-6} M for WGA binding to the low-affinity class of binding sites of wild-type CHO cells (P. Stanley and J. P. Carver, unpublished observation).

The ligands used in this study represent analogues of the distal portion of Asn-linked glycopeptides. When WGA binds to cells, there may be an interaction with other carbohydrate portions in addition to NeuNAc-containing groups. This could be effected through the use of the *secondary* GlcNAc binding site, which, at least in the crystal form of WGA, does not bind NeuNAc residues (Wright, 1980b). In other words, if WGA binding to cells resembles that to oligosaccharides in the crystal, it could be assumed that there exist two NeuNAc specific *primary* sites and two additional *secondary* sites to which GlcNAc-type sugars could bind. The simultaneous occupation of both a *primary* site and a *secondary* site by two portions of a cell surface glycopeptide would result in a great increase in affinity for the reasons discussed above. This hypothesis remains to be tested, since it is unclear whether the spatial orientation of these two sugar binding sites can accommodate this sort of simultaneous binding. A rough prediction of the K_D that would result from such an interaction can be made based on the observed ΔG values of the component parts. The value reported in this work for WGA I binding to N3L (−4.2 kcal/mol) could be representative of the contribution of the antenna of a glycopeptide. The "core" region of Asn-linked glycopeptides, βGlcNAc(1,4)-βGlcNAc(1,*N*)Asn (Montreuil, 1980), resembles the β-(1,4)-linked series of inhibitors of WGA. The ΔG for the binding of βGlcNAc(1,4)βGlcNAc(1,*O*)CH_3 was −6.2 kcal/mol as measured by Lacelle (1979). The sum of these two values affords a ΔG of −10.4 kcal/mol, which corresponds to a room temperature K_D value of 2×10^{-8} M. Affinities of this magnitude have been observed for WGA binding to the intermediate-affinity binding sites of CHO cells (P. Stanley and J. P. Carver, unpublished observation). The predicted ΔG value for an interaction with a glycopeptide terminating in an α(2,6)-linked NeuNAc would be about 1.2 kcal/mol less for WGA I, or approximately 9.2 kcal/mol, which represents a K_D value of 1.5×10^{-7} M at room temperature. The existence of multiple classes of WGA binding sites on the surface of CHO cells (Stanley et al., 1980) may reflect these types of structural permutations of the oligosaccharides to which the lectin is binding.

Acknowledgments

We express our gratitude to Anthony T. Garber for performing the NEPHGE and $NaDodSO_4$–polyacrylamide gel electrophoresis. Helpful discussions with Alexander E. MacKenzie, Dr. Arthur A. Grey, and Dr. Giacomo A. F. d'Agostaro are gratefully acknowledged.

References

Adair, W. L., & Kornfeld, S. (1974) *J. Biol. Chem. 249*, 4696–4704.

Allen, A. K., Neuberger, A., & Sharon, N. (1973) *Biochem. J. 131*, 155–162.

Barondes, S. H. (1980) in *The Cell Surface: Mediator of Developmental Processes* (Subtelny, S., & Wessell, N. K., Eds.) pp 349–363, Academic Press, New York.

Barondes, S. H. (1981) *Annu. Rev. Biochem. 50*, 207–231.

Bassett, E. W. (1975) *Prep. Biochem. 5*, 461–477.

Bhavanandan, V. P., & Katlic, A. W. (1979) *J. Biol. Chem. 254*, 4000–4008.

Briles, E. B., Li, E., & Kornfeld, S. (1978) *J. Biol. Chem. 252*, 1107–1116.

Dahlquist, F. W., & Raftery, M. A. (1968) *Biochemistry 7*, 3269–3277.

Dorland, L., Haverkamp, J., Vliegenthart, J. F. G., Strecker, G., Michalski, J.-C., Fournet, B., Spik, G., & Montreuil, J. (1978) *Eur. J. Biochem. 87*, 323–329.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956) *Anal. Chem. 28*, 350–356.

Finne, J., Tao, T.-W., & Burger, M. M. (1980) *Cancer Res. 40*, 2580–2587.

Finne, J., Burger, M. M., & Prieels, J.-P. (1982) *J. Cell Biol. 92*, 277–282.

Garber, A. T., & Gold, R. J. M. (1982) *Exp. Eye Res.* (in press).

Glasoe, P. K., & Long, F. A. (1960) *J. Phys. Chem. 64*, 188–190.

Goldstein, I. J., & Hayes, C. E. (1978) *Adv. Carbohydr. Chem. Biochem. 35*, 127–340.

Grivet, J.-P., Delmotte, F., & Monsigny, M. (1978) *FEBS Lett. 88*, 176–180.
Jordan, F., Bassett, E., & Redwood, W. R. (1977) *Biochem. Biophys. Res. Commun. 75*, 1015–1021.
Jordan, F., Bahr, H., Patrick, J., & Woo, P. W. K. (1981) *Arch. Biochem. Biophys. 207*, 81–86.
Lacelle, N. (1979) Ph.D. Thesis, University of Toronto, Toronto.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Lanir, A., & Navon, G. (1971) *Biochemistry 10*, 1024–1032.
Marchesi, V. T. (1972) *Methods Enzymol. 28*, 354–356.
Monsigny, M., Roche, A.-C., Sene, C., Maget-Dana, R., & Delmotte, F. (1980) *Eur. J. Biochem. 104*, 147–153.
Montreuil, J. (1980) *Adv. Carbohydr. Chem. Biochem. 37*, 157–223.
Nagata, Y., & Burger, M. M. (1974) *J. Biol. Chem. 249*, 3116–3122.
Nicolson, G. L. (1974) *Int. Rev. Cytol. 39*, 89–190.
O'Farrell, P. Z., Goodman, H. M., & O'Farrell, P. H. (1977) *Cell (Cambridge, Mass.) 12*, 1133–1142.
Peters, B. P., Ebisu, S., Goldstein, I. J., & Flashner, M. (1979) *Biochemistry 18*, 5505–5511.
Rice, R. H., & Etzler, M. E. (1974) *Biochem. Biophys. Res. Commun. 59*, 414–419.
Rice, R. H., & Etzler, M. E. (1975) *Biochemistry 14*, 4093–4099.
Schneir, M. L., & Rafelson, M. E., Jr. (1966) *Biochim. Biophys. Acta 130*, 1–11.
Stanley, P., & Carver, J. P. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5056–5059.
Stanley, P., Sudo, T., & Carver, J. P. (1980) *J. Cell Biol. 85*, 60–69.
Steel, R. G. D., & Torrie, J. H. (1960) in *Principles and Procedures of Statistics*, pp 183–193, McGraw-Hill, New York.
Swift, T. J., & Connick, R. E. (1962) *J. Chem. Phys. 37*, 307–320.
Veh, R. W., Michalski, J.-C., Corfield, A. P., Sander-Wewer, M., Gies, D., & Schauer, R. (1981) *J. Chromatogr. 212*, 313–322.
Wright, C. S. (1980a) *J. Mol. Biol. 139*, 53–60.
Wright, C. S. (1980b) *J. Mol. Biol. 141*, 267–291.
Wright, C. S. (1981) *J. Mol. Biol. 145*, 453–461.

Stability and Components of Mature Simian Virus 40†

Minou Bina,* Sherry Beecher, and Veronica Blasquez

ABSTRACT: We have examined the stability of mature simian virus 40 (SV40) to changes in pH and have investigated the DNA-specific enzymatic activities that are released from SV40 virions. Our studies show that when SV40 virions are disrupted by the alkaline disruption method described by Brady et al. [Brady, J. N., Winston, V. D., & Consigli, R. A. (1977) *J. Virol. 23*, 717–724], in addition to the capsid proteins, a DNA topoisomerase and a DNA endonuclease are released. Treatment with a reducing agent and a divalent ion chelator is required for the release of both enzymes from the mature virions. Kasamatsu and Wu have previously isolated from SV40 virions a protein covalently linked to SV40 DNA under strongly denaturing conditions [Kasamatsu, H., & Wu, M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1945–1949]. Considering the known biochemical properties of DNA topoisomerases, we propose that the protein in Kasamatsu and Wu's isolated protein–DNA complex is the SV40-associated topoisomerase. In this work, we show that the topoisomerase is activated by Mg^{2+} ions and does not require a high-energy cofactor to relax supercoiled DNA. On the other hand, the endonuclease is active at 37 °C only in the presence of Mg^{2+} ions. While the topoisomerase and the endonuclease activities are released from the virions near neutral conditions, the extent of dissociation of capsid proteins from SV40 chromosomes and the shape of subviral DNA–protein complexes depend on pH. At pH 10.4, the DNA-containing particles sediment at 60 S. At lower pH values, 9.8 and 9.2, the subviral nucleoprotein complexes sediment at 75 and 92 S, respectively. Below pH 9.0, the virus particles decay to a heterogeneous mixture of DNA-containing particles. The majority of the nucleoprotein complexes that result from disruption of the virions at pH 8.8 appears as highly compact SV40 chromatin. Electron microscopic observations and biochemical analysis suggest that a fraction of encapsidated SV40 chromatin seems to be deficient in histones or "normal" nucleosomes.

Simian virus 40 (SV40) is a small icosahedral nonenveloped virus. About 88% of the SV40 virion is protein, and the remaining 12% is DNA [reviewed by Tooze (1980), Nathans (1979), and Chambon (1977)]. Highly purified SV40 virions contain at least seven polypeptides. The four minor viral proteins correspond to the cellular histones H2A, H2B, H3, and H4. The three other polypeptides (VP1, VP2, and VP3) are encoded by the SV40 genome (Tooze, 1980).

Within virions and in infected cells, the SV40 DNA is folded by the cellular histones into the SV40 chromosome or minichromosome (Griffith, 1975; Chambon, 1977). The structure of minichromosomes resembles the fundamental structure of eukaryotic chromatin. The DNA is organized into units (nucleosome core), each involving approximately a 146 base pair segment of DNA and an octamer of the four cellular histones (Chambon, 1977). The capsid proteins form an icosahedron of 42-nm diameter encasing the minichromosomes (Tooze, 1980).

The integrity of mature SV40 virions is maintained by pH-dependent bonds, divalent ions, and disulfide bridges. Strong alkaline conditions (pH 10.5) disrupt the SV40 virions (Huang et al., 1972). At pH 9.8 in the presence of a reducing agent, the major capsid proteins dissociate from the SV40 chromosomes; the protein components that remain bound to

† From the Department of Chemistry, Division of Biochemistry, Purdue University, West Lafayette, Indiana 47907. *Received November 13, 1981.* This work was supported by the American Cancer Society.

0006-2960/82/0421-3057$01.25/0