Pober, J. S., & Stryer, L. (1975) *J. Mol. Biol. 95*, 477–481.
Rehorek, M., & Heyn, M. P. (1979) *Biochemistry 18*, 4977–4983.
Reynolds, J. A., & Tanford, C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4467–4477.
Robinson, N. C., & Tanford, C. (1975) *Biochemistry 14*, 369–378.
Sale, G. J., Towner, P., & Aktar, M. (1977) *Biochemistry 16*, 5641–5649.
Sardet, C., Tardieu, A., & Luzzati, V. (1976) *J. Mol. Biol. 105*, 383–407.
Schechter, N. M., Sharp, M., Reynolds, J. A., & Tanford, C. (1976) *Biochemistry 15*, 1897–1904.
Small, D. M. (1971) in *The Bile Acids* (Nair, P. P., & Kritchevsky, D., Eds.) Vol. 1, pp 249–353, Plenum Press, New York.
Smith, H. G., Jr., Stubbs, G. W., & Litman, B. J. (1975) *Exp. Eye Res. 20*, 211–217.
Stryer, L. (1978) *Annu. Rev. Biochem. 47*, 819–846.
Suzuki, T., Makino, M., & Azuma, M. (1977) *Biochim. Biophys. Acta 494*, 115–125.
Tanford, C. (1961) *Physical Chemistry of Macromolecules*, Wiley, New York.
Tanford, C., Nozaki, Y., Reynolds, J. A., & Makino, S. (1974) *Biochemistry 13*, 2369–2376.
Tanford, C., Nozaki, Y., & Rhode, M. F. (1977) *J. Phys. Chem. 81*, 1555–1560.
Wald, G., & Brown, P. K. (1953) *J. Gen. Physiol. 37*, 189–200.
Weber, K., & Osborn, M. (1975) *Proteins, 3rd Ed. 1*, 179–223.
Wright, A. K. (1976) *Biophys. Chem. 4*, 199–202.
Wu, C.-W., & Stryer, L. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1104–1108.
Zacharius, R. M., & Zell, T. E. (1969) *Anal. Biochem. 30*, 148–152.

Comparison of Binding Sites for Wheat Germ Agglutinin on Raji Lymphoblastoid Cells and Their Isolated Nuclei and Plasma Membranes†

Marti Jett* and G. A. Jamieson

ABSTRACT: Raji lymphoblastoid cells and the cell nuclei and plasma membranes isolated by the glycerol-lysis technique [Jett, M., Seed, T., & Jamieson, G. A. (1977) *J. Biol. Chem. 252*, 2134–2142] have been examined for their ability to bind wheat germ agglutinin. Intact cells and isolated nuclei showed similarities (i) in the total number of binding sites (3.38×10^6 and 4.06×10^8, respectively), indicating at least a 2-fold higher receptor density on the nuclei, (ii) in the ratios of the number of high-affinity sites and low-affinity sites (1.05 and 1.07), and (iii) in the apparent association constants at the high-affinity sites (28 nM and 48 nM) and at the low-affinity sites (116 nM and 370 nM). Isolated plasma membranes had a similar number of total binding sites calculated on an equivalent cell basis (2.01×10^6) but showed differences in the ratio of high- to low-affinity sites (1.5) and in their apparent association constants (3 nM and 22 nM). These results suggest similarities in the lectin receptors on the outer surface of lymphoblastoid cells and the cell nuclei. The differences obtained with isolated membranes may be due to inversion of the membrane vesicles or to their decreased rigidity as compared with the intact cell.

The relationship between the plasma and nuclear membranes of cells is not clear but is of obvious importance in understanding membrane biosynthesis, cell growth, and neoplastic transformation. Morphologically, the two membrane systems may be continuous (Grundmann, 1699), and they are known to share enzymes such as 5′-nucleotidase (Crumpton & Snary, 1974; Wallach & Winzler, 1974) and thymidine-5′-phosphodiesterase (Erecinska et al., 1969; Jett et al., 1977).

Lectins have been used extensively to elucidate relationships between the cellular membrane comparments. Fluorescent and ferritin-conjugated lectins such as concanavalin A[1] and wheat germ agglutinin, which bind to intact cells, also bind to bovine liver nuclei (Nicolson et al., 1972) and to rat liver nuclei (Virtanen & Wartiovaara, 1976). Internalization of Con A has been observed with bovine hepatic cells under normal incubation conditions within 1–2 h (Nicolson, 1974). *Ricinus communis* lectin and phytohemagglutinin have been shown to interalize, presumably while bound to their surface receptors, into the GERL of mouse ganglion neurons within 1–3 h upon incubation at 37 °C (Gonatas et al., 1977). The ultimate fate of the internalized lectin is not known in either case. However, quantitative comparisons of the distribution and nature of the lectin binding sites on cells and nuclei are lacking.

WGA binds to surface glycoproteins in a number of different cell types (Nagata & Burger, 1974; Goldstein & Hayes, 1978), and the lectin specifically is directed mainly toward GlcNAc, although AcNeu is about one-half as effective (Peters et al., 1979; Bhavandan & Katlic, 1979). We have now quantitated the binding of wheat germ agglutinin to high- and low-affinity sites on intact Raji lymphoblastoid cells and their isolated nuclei and plasma membranes prepared by this procedure. A preliminary account of some aspects of this work has already appeared (Jett, 1977).

Materials and Methods

Sepharose-bound WGA was obtained from Vector Laboratories (Burlingame, CA). [3H]Glucosamine (173 mCi/mg, uniformly labeled) was obtained from Amersham/Searle (Arlington Heights, IL).

† From the American Red Cross Blood Services Laboratories, Bethesda, Maryland 20014. *Received January 14, 1981.* This investigation was supported, in part, by U.S. Public Health Service Grants HL 20971, HL 14697, and RR05737. Contribution No. 452 from the American Red Cross.

[1] Abbreviations used: Con A, concanavalin A; WGA, wheat germ agglutinin; PBS, phosphate-buffered saline; GlcNAc, *N*-acetylglucosamine; Cl_3CCOOH, trichloroacetic acid; AcNeu, *N*-acetylneuraminic acid.

0006-2960/81/0420-5221$01.25/0

Wheat Germ Agglutinin. WGA was obtained from Calbiochem (La Jolla, CA). It was iodinated with ^{125}I (New England Nuclear, Boston, MA) by the method of Cuatrecasas (1973) and purified by affinity chromatography (March et al., 1974) on ovomucoid–Sepharose (Levine et al., 1972). [^{3}H]WGA (New England Nuclear) was similarly purified by affinity chromatography and used for only up to 10 days from the date of the radiolabeling on the manufacture's recommendation since it had been labeled by the tritium gas exchange technique. The protein content of each preparation was determined from the absorbance at 280 nm by using a standard curve of WGA. The specific activity of the samples was 3.2–6.0 Ci/mmol (188–353 μCi/mg) for the tritiated lectin and 0.72 Ci/mmol (42 μCi/mg) for the iodinated lectin. Similar results were obtained by using either lectin preparation.

Radioactivity Measurements. The radioactive determinations were made by using an Intertechnique liquid scintillation counter (IN/US Corp., Fairfield, NJ). Quench curves were constructed by using ten 1-μCi tritium capsules and, separately, ten 0.45-μCi ^{14}C samples (LKB instruments, Rockville, MD) and adding aliquots of up to 200 μL of carbon tetrachloride as the quenching agent. The quench curves were calculated by published methods (Reunanen & Soini, 1974) for both single-label and double-label experiments. The degree of quench of each sample was determined by using a radium external standard since the channels ratio method was found not to be sufficiently accurate when dealing with the low-energy β emissions of tritium.

Particulate samples containing bound lectin were solubilized by using 300 μL of NCS tissue solubilizer (Amersham) while aqueous samples containing free lectin were emulsified by using NCS solubilizer. The samples were then mixed with 10 mL of toluene–Spectrafluor (1:0.06) (Amersham) to give a single phase. Similar quench curves were found for both the bound and free samples and the counting efficiency varied between 38% and 44%, depending on the degree of quench.

Cell Culture. Raji lymphoblastoid cells were obtained from American Type Culture Collection (ATCC; Rockville, MD) as frozen stock cultures at passage 110 and had been characterized by ATCC as being virus free. The cells were cultured in 150-cm^2 Costar plastic tissue culture flasks (Bellco, Vineland, NJ) with Eagle's minimum essential medium for suspension cultures (Microbiological Associates, Walkersville, MD) supplemented with 12% fetal calf serum, which had been heat inactivated at 56 °C for 30 min (GIBCO, Grand Island, NY), 2% kanamycin, and 2% glutamine (Microbiological Associates). Cultures were examined daily for any signs of alteration in growth characteristics and were discontinued after 20–30 passages, and new cultures were established from frozen stock. In some experiments, cells (1.2×10^9) in 150 mL of culture medium were labeled with [^{3}H]glucosamine (50 μCi) 48 h after subculture, and the cells were harvested 18 h later.

Preparation of Cell Fractions. Whole cells were harvested aseptically by transferring the cultures to sterile 250-mL plastic conical centrifuge bottles (Corning Glass Co., Corning, NY) and centrifuging at 27 °C in a Sorvall RC4 centrifuge (Du Pont/Sorvall, Newton, CO) using an HG-4L rotor at 2000 rpm [800g (average)] for 10 min, and the cells were washed once with warm PBS. Cells were disrupted by the hypotonic glycerol lysis technique, and nuclei, plasma membranes, and other cell fractions were prepared as previously described (Jett et al., 1977). The intact cells and isolated nuclei were examined by phase contrast microscopy using a Zeiss light microscope to ascertain that the organelles were intact, unclumped, and of similar appearance to other preparations. Concentrations of intact cells and isolated nuclei were also determined with the same microscope at a magnification of 625× using a hemocytometer.

In these experiments, nuclei were isolated just prior to use since their preparation was simple, although they are stable at 4 °C for at least several days and substantially longer when isolated and handled by using sterile procedures.[2] Plasma membranes were also prepared fresh daily since freezing and thawing resulted in some clumps which required sonication for disruption, and this might cause membrane changes.

Lectin Binding Assay. The standard assay system was developed on the basis of the studies on the kinetics of binding as described under Results. In the standard assay, the concentration of the cell fraction was adjusted so that the lowest levels of bound lectin would be approximately 5000 dpm. In a typical experiment, intact cells [$(2–3) \times 10^6$] and 0.01–10 μg of radiolabeled lectin in a total volume of 210 μL of PBS were incubated at ice bath temperature for 1 h in 1.6-mL Eppendorf microcentrifuge tubes. One hundred microliters of the reaction mixture was then quickly layered onto 200 μL of a 1:3 (v/v) mixture of Apiezon oil (Apiezon Products Ltd., London) and Dow Corning 704 diffusion pump fluid (Beckman Corp., Palo Alto, CA) contained in a 400-μL Eppendorf microcentrifuge tube (Brinkmann Instruments, Westbury, NY) which was chilled at 4 °C to prevent inversion of the oil and aqueous layers as warming occurred during subsequent centrifugation (12000g, 3 min) in an Eppendorf tabletop microcentrifuge. The aqueous phase was then removed by using a disposable 1-mL tuberculin syringe (Monoject, Fisher Chemical Co., Silver Spring, MD) with an 18-gauge needle and placed in a liquid scintillation vial. The oil layer did not contain radioactivity but was removed separately to avoid cross-contamination and added to the aqueous phase. The pelleted cells were then dissolved in NCS tissue solubilizer and the tubes washed with several 50-μL aliquots of NCS. Nonspecific or irreversible binding of WGA was determined by adding 10μL of 1.2 M GlcNAc (Sigma, St. Louis, MO) to the remaining 110 μL of incubation mixture, removing another aliquot of 100 μL, and processing as described above.

In experiments using isolated nuclei or plasma membranes, the amounts chosen for the standard assay system were 2.9×10^6 nuclei and 55 μg of plasma membrane protein as determined by the Folin assay (Folin & Ciocalteu, 1927), and centrifugation (12000g) was carried out for 5 and 7 min, respectively.

At neutral pH, WGA forms a dimer of 34 000 daltons (Nagata & Burger, 1974; Goldstein & Hayes, 1978). Each single polypeptide chain has two binding sites for GlcNAc, but it is assumed that only one of these needs to be filled in order to get binding to the cell fraction. Thus, calculations for the number of binding sites reported in this study assume a univalent molecular weight of 17 000 for WGA.

Results

Kinetics of Binding. In general, the time course of lectin binding was similar for all three cell fractions, with maximum uptake by 60 min and only an additional 2% uptake occurring with further incubation up to 90 min in the case of intact cells.

Reversal of binding by GlcNAc was instantaneous whether the inhibitor was added before the addition of WGA to the cell fraction, immediately after, or at various times during the incubation up to 90 min. Furthermore, the amount of nonreversible binding was unaffected by the length of incubation up to 60 min, but gradually increased beyond that time.

[2] Observations to be published elsewhere.

Table I

	high-affinity sites [nmol bound/μg of protein (×10⁴)]	total nmol bound/μg of protein (×10⁴)	ratio of high/low affinity sites	total nmol bound/cell (×10⁹)	total no. of binding sites/cell	app association constant (nM)	
						high	low
whole cells							
range	0.213–0.327	0.429–0.578		5.56–5.69	3.38 × 10⁶	27.6	116
average	0.276 ± 0.035 (8)[a]	0.538 ± 0.074 (4)	1.05	5.61 ± 0.037			
nuclei							
range	0.72–0.78	1.33–1.62		6.61–6.89			
average	0.75 (2)	1.45 ± 0.149 (3)	1.07	6.74 ± 0.115	4.06 × 10⁶	48	370
plasma membrane							
range	9.08–10.24	14.8–15.7					
average	9.82 ± 0.391 (6)	15.32 ± 0.388 (4)	1.51		2.01 × 10⁶ [b]	3.36	22

[a] The number in parentheses represents the number of experiments used in obtaining the average. [b] Assuming that 95 mg of whole cell protein gives rise to 2.16 mg of plasma membrane protein (Jett et al., 1977).

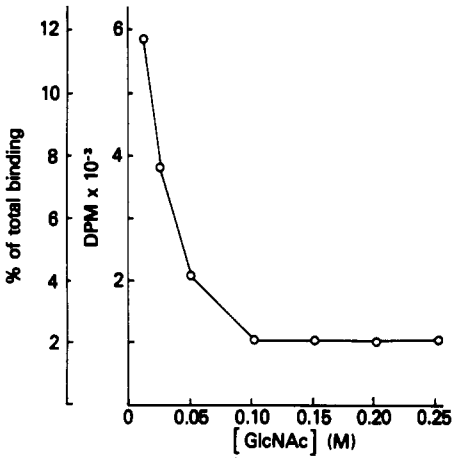

FIGURE 1: Determination of the optimal concentration of GlcNAc to inhibit WGA binding. Intact cells (1.82×10^6) were incubated with 100 nM WGA for 1 h at 4 °C after which GlcNAc was added to give final concentrations ranging from 0.01 to 0.25 M, as shown. WGA subunit mol wt 17 000.

Methyl α-mannoside and galactose at similar concentrations did not show any inhibition.

Increasing concentration of GLcNAc inhibited the binding of WGA to Raji lymphoblastoid cells, with maximum inhibition being reached at 0.1 M concentration (Figure 1), similar to the value found in other cell systems (Cuatrecasas, 1973; Ozanne & Zambrook, 1971). Microscopically obvious toxicity to the cells and nuclei was detected at concentrations of 0.18 M. The residual radioactivity bound to the cells after exposure to GlcNAc is considered to be due to irreversible or nonspecific binding and was equal to 2% of the total bound disintegration per minute for intact cells, 4.5% for isolated nuclei, and 2% for plasma membranes at the cell/lectin ratio showing the maximum percentage of binding under the conditions of the standard assay system.

The percentage of the binding which was not reversed by the hapten sugar varied from 1.8% to 9.7% for intact cells, depending on the concentration of lectin incubated (Figure 2A). Similar variations occurred for both nuclei and plasma membranes (Figure 2B,C). The lowest degree of nonreversible binding occurred at the lectin concentration showing maximum specific binding. However, this irreversible or nonspecific binding increased at very low and at high lectin concentrations. Therefore, the standard assay was designed so that the test for total and nonreversible binding was carried out on the same incubation sample in order to permit determination of the specifically bound WGA. Binding was compared at 4 and 25 °C; since the nonreversible binding was nearly twice as high at 25 °C as that at 4 °C, all subsequent experiments were

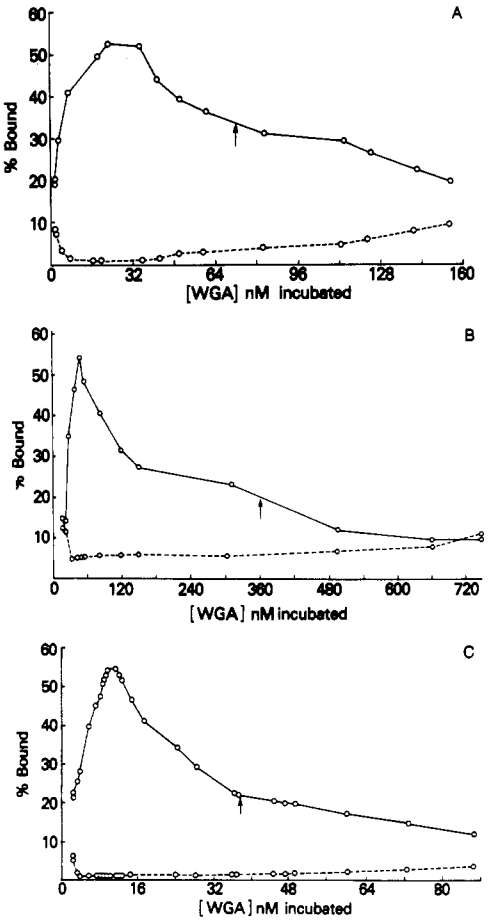

FIGURE 2: Percentages of total WGA bound to the cell fractions in the presence and absence of 0.1 M GlcNAc. (O—O) Bound WGA dissociable by GlcNAc. (O---O) Percentage of the total bound WGA which is not dissociable by GlcNAc. (A) Intact cells; Each assay point contained 1.46×10^6 cells/100 μL. (B) Nuclei, 1.31×10^6/100 μL. (C) Plasma membranes, 26.2 μg/100 μL. The arrows show the points at which the slope change occurs in the Scatchard plots (Figure 3) of these data. WGA subunit mol wt 17 000.

conducted at the lower temperature.

Direct and Scatchard Plots of the Data. The graphical analysis of the data for each of the cell fractions examined is presented in Figure 3 and is summarized in Table I.

(*i*) *Intact Cells.* The binding of WGA was biphasic (Figure 3A), showing sigmoid curvature at lectin concentrations up to 8 nM (0.14 μg/mL). The Scatchard plot (Figure 3B) shows, in detail, the typical cooperative binding, with the Dahlquist maximum (Dahlquist, 1978) occurring at approximately 0.08×10^{-4} nmol bound/μg of protein. The slope change in the Scatchard plot occurs at an incubation con-

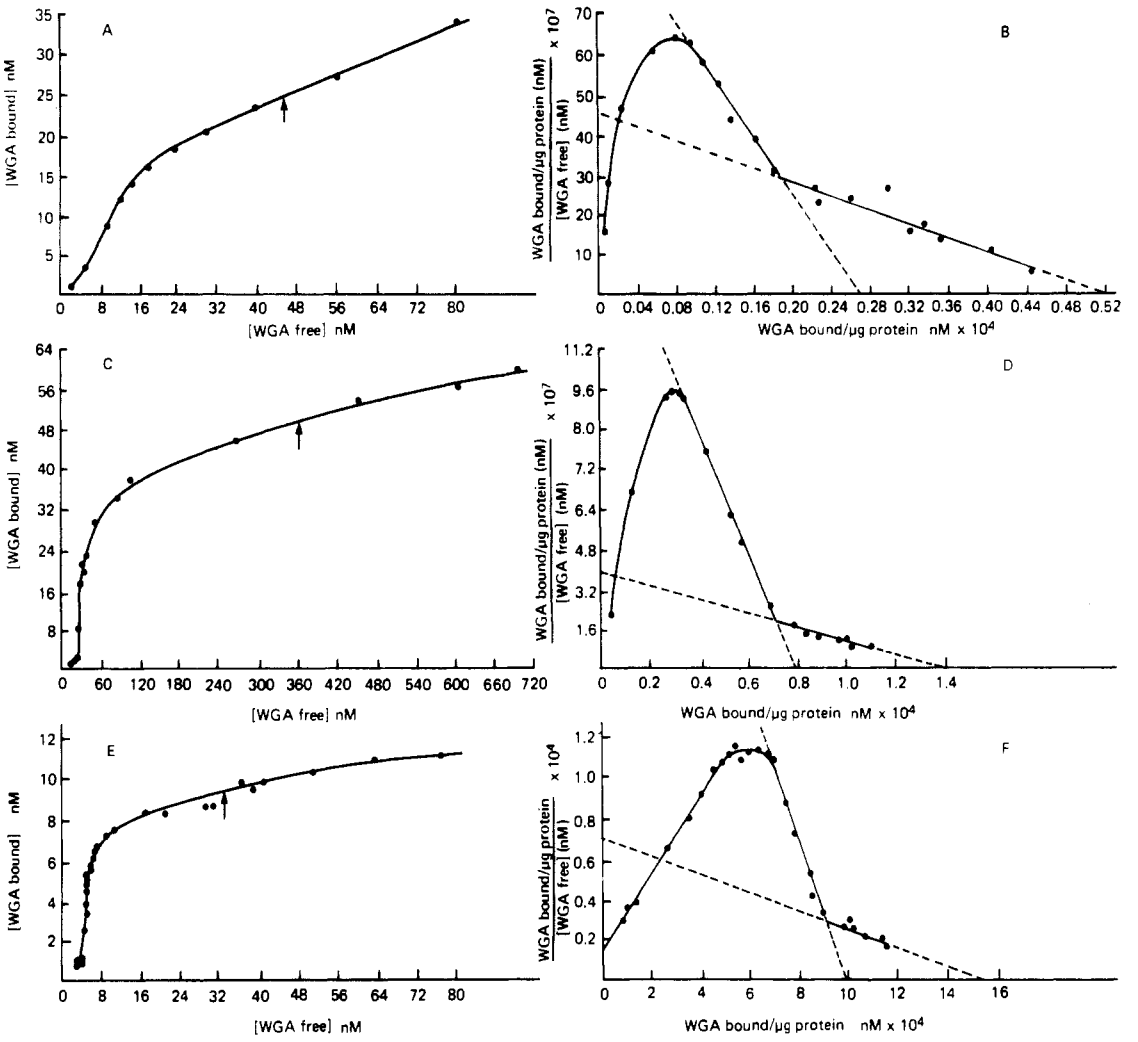

FIGURE 3: Binding of WGA to cells and cell fractions. (A, C, and E) Direct plots of the data as the concentration bound (nM) vs. the concentration free (nM). The arrows indicate the points at which there is a slope change on the Scatchard plots. (B, D, and F) Scatchard plots. The broken lines are extrapolations to the abscissa in order to estimate the number of binding sites. Intact cells (A, B), 1.46 × 10^6 cells (152.38 μg of protein) per 100 μL; nuclei (C, D), 1.314 × 10^6 nuclei (68.25 μg of protein) per 100 μL; plasma membranes (E, F), 26.19 μg of protein of isolated membranes per 100 μL. WGA subunit mol wt 17 000.

centration of 70 nM (1.2 μg/mL), and extrapolation of the binding curves to the abscissa showed essentially equal amounts bound at high- and low-affinity binding sites, 0.276 × 10^{-4} and 0.262 × 10^{-4} nmol/μg of cell protein, respectively, corresponding to 1.73 × 10^6 and 1.65 × 10^6 high- and low-affinity sites per cell, respectively. The apparent association constants of the high- and low-affinity sites were 28 nM and 116 nM, a 4-fold difference.

(*ii*) *Isolated Nuclei.* In the case of isolated nuclei, there was a similar biphasic binding up to a WGA concentration of 26 nM (0.44 μg/mL) (Figure 3C), with the maximum percentage of binding occurring at a concentration of 60 nM (Figure 2B). Scatchard plots (Figure 3D) showed a slope change at WGA concentration of 383 nM, with similar amounts being bound at high- and low-affinity sites of 0.78 × 10^{-4} and 0.62 × 10^{-4} nmol of WGA/μg of nuclear protein with association constants of 48 and 370 nM, respectively (Table I). These figures correspond to 2.11 × 10^6 and 1.95 × 10^6 high- and low-affinity sites per nucleus.

(*iii*) *Plasma Membranes.* Direct plots of binding to isolated plasma membranes showed significant evidence of cooperativity up to a lectin concentration of 4 nM (0.07 μg/mL) (Figure 3E), with the maximum percentage binding at 12 nM (Figure 2C). The Scatchard plots (Figure 3F) showed the slope change occuring at 32 nM (0.55 μg/mL) and gave values for binding at the high- and low-affinity sites of 9.5 × 10^{-4} and 6.5 × 10^{-4} nmol of WGA/μg of membrane protein, corresponding to 1.29 × 10^6 and 0.72 × 10^6 high- and low-affinity sites per equivalent cell, respectively. The association constants at these high- and low-affinity sites were 3 nM and 22 nM, a 7-fold difference.

Discussion

Questions have been raised regarding the nature, number, and distribution of lectin receptors on nuclei in a variety of diffeent cells, particularly with regard to the lectin-induced transformation of lymphoblastoid cells. The techniques described here have furnished accurate quantitation of the binding of WGA to Raji lymphoblastoid cells, to cell nuclei, and to isolated plasma membranes and have given some insights as to the chemical nature of the receptors. Particular attention was paid to methods for separating bound from free lectin. Cells have been separated from the lectin incubation medium by filtration or centrifugation are contaminated with residual unbound lectin, but washing to remove this contamination may disrupt the equilibrium that has been attained. Similarly, the use of step gradients of sucrose or 20% fetal calf serum, as has been used in certain lectin binding studies (Greene, 1976), was considered undesirable since the high carbohydrate and glycoprotein content of these solutions might cause dissociation of the lectin from the cell fraction and so

disturb the equilibrium. The use of a mixture of oils of different densities, as has been used in binding studies with platelets (Martin et al., 1976), seemed to be free of these disadvantages.

The concentration dependence of the binding of WGA was generally similar for intact cells and their isolated nuclei and plasma membranes with an initial sigmoidal shape indicative of cooperative interactions; positive cooperativity has also been observed in the binding of WGA to intact Chinese hamster ovary cells (Stanley & Carver, 1977; Stanley et al., 1980). In the present work, the percentage of bound lectin that was dissociable by GlcNAc (specific binding) followed a typical pattern, with the maximum binding occurring over a limited range. The three cell fractions examined showed similar patterns, with the percentage specifically bound rising rapidly with small increments in lectin concentrations. The portion of the Scatchard plots showing cooperative interactions occurs at these very low lectin concentrations, while the Scatchard high-affinity sites are determined at the lectin concentrations showing the maximum percentage of specific binding. The Scatchard determination of the total number of binding sites corresponds to higher lectin concentrations in which the percentage specifically bound shows a gradual decrease to approximately 12%.

Bound lectin which is not dissociable by the haptenic sugar is frequently referred to as nonspecific binding. The nature of this binding is not clear, and it may differ between the very lowest and the highest lectin concentrations. The first few lectin molecules bind to the receptors with very high affinity so that this may represent an irreversible binding, at least with the low molecular weight haptenic sugars used. The percentage of bound lectin that is not dissociable by the haptenic sugar steadily increases at higher lectin concentrations and may be due to lectin aggregates which have a higher affinity for the surface or even to internalization of lectin molecules. The importance of evaluating binding at low lectin concentrations due to biological considerations has been stressed (Reisner et al., 1976), and the present data emphasize the need for determining both total and nonreversible binding at each lectin concentration used. This problem is particularly marked in the case of the binding of Con A in which nonreversible binding can account for 72% of total binding at low lectin concentrations.[2]

The total number of specific binding sites on intact cells was less variable from different experiments when calculated on the basis of moles bound per cell rather than moles bound per microgram of protein, due to the fact that the latter ranged from 87 to 109 $\mu g/10^6$ cells. Although the cells were always harvested in the log phase of growth, this may represent shifts in proportions of the cells in various stages of the cell cycle.

The total number of binding sites on cells and nuclei were similar, 3.38×10^6 and 4.06×10^6. Electron photomicrographs have shown that the diameter of the isolated nucleus is about 12 μm while that of the intact cell is about 18 μm (Jett et al., 1977) and thus should have over twice the surface area if a spherical configuration is assumed in each case. For the isolated plasma membranes, a value of 2.01×10^6 receptor sites was calculated on an equivalent cell basis. These results show that 63% of the total external lectin binding sites found on intact cells are accounted for in the isolated plasma membrane. This is in good agreement with the estimated yield of 70% of plasma membranes based on enzymatic markers (Jett et al., 1977).

The amount of WGA bound on intact Raji cells was almost equal at the high-affinity and low-affinity binding sites (0.276×10^{-4} and 0.262×10^{-4} nmol/μg of protein, respectively), corresponding to 1.73×10^6 high-affinity and 1.65×10^6 low-affinity sites. In contrast, the numbers of high- and low-affinity binding sites for Con A on intact fibroblasts differ by more than 2 orders of magnitude, being 8×10^5 and 3×10^8 sites per cell, respectively (Feller et al., 1977). In the present work, nearly equal amounts of WGA were bound at high- and low-affinity sites in the case of isolated nuclei (0.75×10^{-4} and 0.70×10^{-4} nmol/μg of protein, respectively), but the values differed somewhat for plasma membranes (9.82×10^{-4} and 6.5×10^{-4} nmol/μg of protein, respectively). These values correspond to 2.11×10^6 high-affinity and 1.95×10^6 low-affinity sites in intact nuclei and 1.29×10^6 and 0.72×10^6 sites, respectively, in isolated plasma membranes calculated on an equivalent cell basis.

The association constants for high-affinity binding were almost 10-fold greater in isolated plasma membranes than in intact cells and about 5-fold greater for low-affinity binding; in fact, the low-affinity binding of isolated membranes (K_a = 22 nM) was similar to the high-affinity binding for intact cells (K_a = 28 nM). Isolated cell membranes frequently appear to form "inside out" vesicles (Steck et al., 1970; Walsh et al., 1976a). Since these vesicles are permeable to unconjugated lectins (Walsh et al., 1976b), WGA may be binding to glycoproteins on the interior concave surface of the vesicle in the case of the plasma membrane preparation, rather than on the exterior convex surface, as is the case for intact cells and nuclei. This, and the relatively lesser rigidity of isolated membranes, might explain the increased cooperative interactions in binding.

Similar considerations may apply with regard to the studies using isolated nuclei. Despite their size, ferritin–lectin complexes can gain access to the cisternal surfaces presumably due to slight breaks in the outer nuclear membrane of rat liver nuclei (Virtanen & Wartiovaara, 1976), amphibian oocyte nuclei (Feldherr et al., 1977), and Raji cells (Jett, 1977) without further disruption. Thus the unconjugated lectins might be expected to bind readily to these receptors, although they are not truly on the outer surface of the nucleus.

In summary, these results show that there are quantitative similarities between the cell surface membranes of Raji lymphoblastoid cells and intact nuclei in their ability to bind WGA. They are nearly identical in (a) the total number of binding sites (3.38×10^6 and 4.06×10^6), although there is a lower receptor density on the intact cell surface because of its greater area, (b) the number of high-affinity sites (1.73×10^6 and 2.11×10^6) and low-affinity sites (1.65×10^6 and 1.95×10^6), and (c) the association constants at these sites, 28 nM and 48 nM for high-affinity sites in the intact cells and the nuclei and 116 nM and 370 nM at the low-affinity sites. Taken together, these data suggest considerable quantitative similarities in the nature of the WGA receptors on the plasma and nuclear membranes of Raji cells and support the idea that the nuclear membrane is the biosynthetic precursor of the plasma membrane of the intact cell.

Acknowledgments

We are grateful to Susan Spielberg for her skillful technical assistance and to Dr. David Kosow for his discussions on the kinetics of the ligand binding.

References

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

Crumpton, M. J., & Snary, D. (1974) *Contemp. Top. Mol. Immunol. 3*, 27–55.

Cuatrecasas, P. (1973) *Biochemistry 12*, 1312–1329.

Dahlquist, F. W. (1978) *Methods Enzymol. 48*, 270–299.
Erecinska, M., Sierakowska, H., & Shugar, D. (1969) *Eur. J. Biochem. 11*, 465–471.
Feldherr, C. M., Richmond, P. A., & Noonan, K. D. (1977) *Exp. Cell Res. 107*, 439–444.
Feller, M., Richardson, C., Behnke, W. D., & Gruenstein, E. (1977) *Biochem. Biophys. Res. Commun. 76*, 1027–1035.
Folin, O., & Ciocalteu, V. (1927) *J. Biol. Chem. 73*, 627–650.
Goldstein, I. J., & Hayes, C. E. (1978) *Adv. Carbohydr. Chem. Biochem. 35*, 127–340.
Gonatas, N. K., Kim, S. U., Stieber, A., & Avrameas, S. (1977) *J. Cell Biol. 73*, 1–13.
Greene, W. C., Parker, C. M., & Parker, C. W. (1976) *J. Biol. Chem. 251*, 4017–4025.
Grundmann, E. (1966) in *General Cytology: An Introduction to Function and Morphology of the Cell*, p 242, Williams and Wilkins, Baltimore, MD.
Jett, M. (1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36*, 2313.
Jett, M., Seed, T., & Jamieson, G. A. (1977) *J. Biol. Chem. 252*, 2134–2142.
LeVine, D., Kaplan, M. J., & Greenaway, P. J. (1972) *Biochem. J. 129*, 847–856.
March, S. C., Parikh, I., & Cuatrecasas, P. (1974) *Anal. Biochem. 60*, 149–152.
Martin, B. M., Wasiewski, W. W., Fenton, J. W., II, & Detwiler, T. C. (1976) *Biochemistry 15*, 4886–4893.
Nagata, Y., & Burger, M. M. (1974) *J. Biol. Chem. 249*, 3116–3122.
Nicolson, G. L. (1974) *Int. Rev. Cytol. 39*, 89–190.
Nicolson, G., LaCorbiere, M., & Delmonte, P. (1972) *Exp. Cell Res. 71*, 468–472.
Ozanne, B., & Sambrook, J. (1971) *Nature (London) 232*, 156–160.
Peters, B. P., Ebisu, S., Goldstein, I. J., & Flashner, M. (1979) *Biochemistry 18*, 5505–5511.
Reisner, Y., Lis, H., & Sharon, N. (1976) *Exp. Cell Res. 97*, 445–448.
Reunanen, M. A., & Soini, E. J. (1974) *Liq. Scintill. Counting 3*, 86–93.
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.
Steck, T. L., Weinstein, R. S., Straus, J. M., & Wallach, D. F. H. (1970) *Science (Washington, D.C.) 168*, 255.
Virtanen, I., & Wartiovaara, J. (1976) *J. Cell Sci. 22*, 355–364.
Wallach, D. F. H., & Winzler, R. J. (1974) in *Evolving Strategies and Tactics in Membrane Research*, Springer-Verlag, New York.
Walsh, F. S., Barber, B. M., & Crumpton, M. J. (1976a) *Biochemistry 15*, 3557–3563.
Walsh, F. S., Barber, B. H., & Crumpton, M. J. (1976b) *Bicohem. Soc. Trans. 4*, 251–252.

Structure, Assembly, Conformation, and Immunological Properties of the Two Subunit Classes of Ferritin†

Shinobu Otsuka,‡ Hiroshi Maruyama, and Irving Listowsky*

ABSTRACT: The two subunit types of human liver ferritin were purified to homogeneity. Both subunits reassembled in a well-defined manner and formed spherical particles that resembled natural apoferritin in electron micrographs. Affinity chromatography methods were employed to obtain preparations of antibodies that interacted exclusively either with the H or with the L polypeptides, demonstrating that distinct immunological properties may be ascribed to each subunit of ferritin. The amino acid compositions of the subunits were similar, but the larger H subunit had fewer leucine, phenylalanine, and arginine residues. It is therefore improbable that H subunits undergo proteolytic processing and are precursors for L subunits. Circular dichroism data indicated that homopolymers assembled from L-type subunits had substantially more ordered secondary structures and greater α-helical contents than their H counterparts. Small differences in the environment of tryptophan residues were evident from fluorescence spectra of each homopolymer. In isoelectric focusing experiments reassembled H or L homopolymers migrated as families of proteins within discrete p*I* ranges which are probably representative of subpopulations of each subunit type. The H homopolymer focused at lower p*I*'s than the L component. These data substantiate the contention that both subunits are authentic polypeptide moieties of ferritin with some common structural features, but the results also underscore prominent dissimilarities in their properties.

Ferritin has 24 subunits arranged in the form of a hollow sphere which envelops an iron micelle in its central cavity (Crichton, 1973; Harrison et al., 1977; Drysdale et al., 1977; Richter, 1978; Munro & Linder, 1978; Aisen & Listowsky, 1980). The protein is found in most mammalian tissues and is synthesized in response to iron (Drysdale & Munro, 1966; Linder-Horwitz et al., 1969; Fagard & Saddi, 1977). It provides a mobilizable reserve of iron in a nontoxic form for the organism's requirements. Ferritins from different organs of a single species may have common peptide sequences but often exhibit small differences in electrophoretic mobility, amino acid composition, and peptide map patterns (Linder & Munro, 1973; Crichton et al., 1973; Bomford et al., 1977; Massover, 1978; Alpert et al., 1979; Kohgo et al., 1980). In addition, a purified ferritin preparation from a single source may consist of a family of closely related proteins ("iso-

† From the Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461. *Received October 22, 1980.* This work was supported by Grants HL 11511 and AM 17702 from the National Institutes of Health.

‡ Present address: Department of Medicine, Cancer Research Institute, Sapporo Medical College, Sapporo, Japan.

0006-2960/81/0420-5226$01.25/0