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ISOLATION OF MEMBRANE GLYCOPROTEINS BY AFFINITY CHROMATOGRAPHY IN THE PRESENCE OF DETERGENTS

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SUMMARY

Wheat germ agglutinin has been used in a one-step preparative method to isolate the major sialoglycoprotein (glycophorin A) from the human erythrocyte membrane. The conditions for isolation and purification of the sialoglycopeptide included low concentration of sodium dodecyl sulfate in the presence of relatively high salt concentration. This medium caused complete solubilization of the membrane but still allowed almost quantitative binding of the sialoglycopeptide to wheat germ agglutinin-Sepharose. The eluted protein from such affinity systems was found to be chemically comparable to glycophorin A, as prepared by other procedures.

INTRODUCTION

Plant lectins have gained considerable prominence [1] as probes for membrane structure [2, 3] and as tools to study differences between normal and transformed cells [4] and the membrane events associated with mitogenesis of lymphocytes [5]. Much of their attraction is derived from the great specificity of some of the lectins for mono- and oligosaccharides and the apparent homogeneity of their saccharide-binding sites. In addition, many of these proteins can be prepared in pure form in high yields by simple procedures [1].

Much less is known about the putative receptor sites on cell membranes for these sugar-binding proteins, in terms of their number, topographical relationships and potential mobility, and even less is known about these receptors in terms of their chemical nature.

Lectins bound to an insoluble matrix have been used as affinity systems with or without detergents for the isolation of these receptors from various cells, including erythrocytes [6], lymphocytes [7, 8], platelets [9], mouse L-1210 cells [10] and mouse L-929 cells [11].

In most of these studies the lectin affinity systems were used to isolate macro-

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molecules of unknown composition and thus there was little opportunity to assess the effects of different experimental conditions in terms of the quality and quantity of the final products. We were particularly interested in developing a method suitable for use in the presence of detergents, since the latter would be needed to solubilize and purify intrinsic membrane proteins. The human red cell membrane and the sialoglycoprotein glycophorin were chosen as the model system to begin this work. Red cell membranes contain a number of intrinsic proteins [12], which require solubilization by detergents, and the most prominent of these (i.e. glycophorin and the band 3 polypeptide) clearly have important receptor functions.

MATERIALS AND METHODS

Materials

Reagent or equivalent grade chemicals were used throughout [35]. Lithium diiodosalicylate was prepared from diiodosalicylate (Eastman Kodak, 2166), twice recrystallized from anhydrous methanol, according to Marchesi and Andrews [24]. Lubrol PX was purchased from Sigma, L-3753, Triton X-100 was from New England Nuclear, NEF-936, and sodium deoxycholate from Fisher Scientific Co., S-285. Hexadecyltrimethylammonium bromide (Eastman Kodak, T 5650) was recrystallized from warm ethanol/ethylacetate (1 : 9, v/v). Dodecyltrimethylammonium bromide was a generous gift from Jordan Pober and was synthesized according to Hong and Hubbell [36] and crystallized from warm ethanol/acetone (15 : 85, v/v). Sodium dodecyl sulfate was obtained from Alcolac Chem. Corp.

Purification of plant lectins

Wheat germ agglutinin was purified directly from raw wheat germ purchased at a local natural food store, by modification of the procedure described earlier [13]. Wheat germ (150 g) was suspended in one liter of cold 0.25 M NaCl in 0.05 M sodium phosphate buffer, pH 7.2, (Buffer A) and homogenized in a Waring blender for 1.5 min at maximum speed. The homogenates from a total of 600 g wheat germ were centrifuged for 10 min at $2300 \times g$. The supernatant was heated for 15 min at 56 °C, cooled to 10 °C and centrifuged as above. The supernatant was filtered through cheesecloth to remove floating fat and the filtrate was absorbed batchwise on 300 ml of an ovomucoid-Sepharose 4B conjugate (see below) for 30–45 min, with casual gentle mixing. The beads were washed extensively with buffer A to $A_{280} < 0.1$, packed in two columns (40×2.2 cm) and were further washed to $A_{280} < 0.01$. The wheat germ agglutinin was eluted with 0.1 M acetic acid and the peak with hemagglutinating activity was dialyzed for 4–16 h successively against 0.01 M acetic acid, 0.5 M NaCl and buffer A for repurification on fresh ovomucoid-Sepharose 4B. The yield of wheat germ agglutinin was about 200 mg/kg of raw wheat germ. The sugar specificity, amino acid analysis and electrophoretic mobility were found to be as expected [14, 15]. Concanavalin A was purified as described by Agrawal and Goldstein [16], *Ricinus communis* agglutinin as described by Tomita et al. [17], and red kidney bean agglutinin as described by Kornfeld et al. [18]. The leuko-agglutinating phytohemagglutinin and the erythroagglutinating phytohemagglutinin were separated as originally described by Weber et al. [19].

Conjugation of lectin to Sepharose 4B

The procedure of Cuatrecasas [20] was modified as follows. 150 ml washed and defined Sepharose 4B beads were suspended in 150 ml 0.2 M $NaHCO_3$ and were activated with 7.5 g of cyanogen bromide at 20 °C for 3 min at pH 11–11.5 (pH adjusted with 4 M NaOH). The beads were washed rapidly with 1.5 l of cold 0.2 M $NaHCO_3$ and the lectin solution (280 mg in 150 ml 0.2 M $NaHCO_3$) was mixed with the beads and placed overnight at 4 °C on a rotatory shaker; the beads were then washed with 2–3 l of 0.2 M $NaHCO_3$ and 1 l of 0.1 M glycine. After incubation at 4 °C in the same buffer for 16–20 h, the beads were washed in 3 l of cold 0.25 M NaCl containing 0.025 % sodium azide in 0.015 M sodium phosphate buffer, pH 7.2, (buffer B) and were stored at 4 °C.

Labeling of intact red blood cells

Freshly drawn red blood cells, collected in citrate/dextrose anticoagulant solution, were washed 3 times in phosphate-buffered saline. Sialic acid residues were tritiated after mild oxidation with periodic acid and successive reduction with NaB^3H_4 (New England Nuclear) as described by Blumenfeld et al. [21]. The enzymatic iodination procedure using lactoperoxidase and glucose oxidase was as described by Segrest et al. [22].

Red blood cell ghosts were prepared from freshly drawn blood as described by Dodge et al. [23], as modified by Fairbanks et al. [27], and Furthmayr and Marchesi [35]. Large batches were processed using a zonal rotor (Beckman J-C2 for a J-21 centrifuge) for continuous flow operation and a density gradient technique. Washed red blood cells from three units of blood were lysed at or below 4 °C in 20 l of 5 mM phosphate buffer (pH 8.0) containing 5.22 mg phenylmethylsulfonylfluoride (Sigma, P-7626)/l as a protease inhibitor. The rotor was loaded at 3000 rev./min with 250 ml of 45 % sucrose (w/w) at a flow rate of 50–100 ml/min from a container pressurized at 5 psi with N_2. The rotor speed was increased to 18 000 rev./min with continuous flow of lysing buffer. Flow of the lysate was started at a rate of up to 170 ml/min. The membrane band was collected after reducing the rotor speed to 2000 rev./min by displacement with 50 % sucrose (w/w). This procedure separated the red blood cell membranes grossly from any residual contaminating white blood cells. After emptying the rotor completely, the entire procedure was repeated once more and the final membrane suspension was washed twice with a total of 3.5 l of 25 mM Tris · HCl (pH 8.0) by centrifugation in a fixed angle rotor (JA-14) at 14 000 rev./min for 20 min. Residual sucrose was not found to interfere with the extraction method used to isolate the sialoglycoprotein fraction (see below).

Extraction and iodination of human erythrocyte membrane sialoglycoprotein

The sialoglycoprotein fraction was extracted from red blood cell ghosts using the lithium diiodosalicylate procedure as described by Marchesi and Andrews [24] and was iodinated as above.

Binding of glycophorin to wheat germ agglutinin-sepharose beads

Wheat germ agglutinin-Sepharose beads were washed with 0.1 M NaCl in 0.05 M sodium phosphate (pH 7.2) by centrifugation for 5 min at $1000 \times g$ and were suspended to 50 % by volume in phosphate-buffered saline with or without carbo-

hydrates. Bead samples (usually 1 ml) were incubated in 13 × 100 mm tubes for 30 min at room temperature with 50–200 μg/100 μl of ^{125}I-labeled human red blood cell membrane glycoprotein, and the bound ^{125}I was estimated in a Beckman gamma-counter, model Biogamma after washing 3 times with a total of 10–20 ml of phosphate-buffered saline.

Purification of glycophorin A by affinity chromatography on wheat germ agglutinin-Sepharose 4B

Membranes were solubilized in 1 % sodium dodecyl sulfate in buffer B (2–4 mg protein/ml) and incubated for 15 min at 37 °C. The solution was centrifuged for 15 min at 48 000 × *g*. A small amount of material was pelleted. The supernatant was diluted 1 : 20 in buffer B and filtered through a wheat germ agglutinin-Sepharose 4B column (2.5–5 ml beads/10 mg membrane protein). The column was washed with 0.05 % sodium dodecyl sulfate in buffer B to an A_{280} of < 0.005. Elution of the bound material was done with 0.1 M *N*-acetyl-D-glucosamine in 0.05 % sodium dodecyl sulfate in buffer B. Occasionally, the column was kept for 1 h in this buffer, before elution was done. To prevent crystallization of sodium dodecyl sulfate and/or salt, the elution buffer had to be kept at or above 25 °C. Stirring on a stirring motor was found to be convenient and sufficient. The fractionation was followed by determination of the absorbance at 280 nm and radioactivity tracing. The fractions were dialyzed for 16 h at room temperature against distilled water. Wheat germ agglutinin-Sepharose beads were regenerated by washing with 1–2 column volumes of 2 % sodium dodecyl sulfate in buffer B and 10–20 volumes of 0.05 % sodium dodecyl sulfate in the same buffer, and could be used for several experiments without appreciable loss of binding capacity.

Analytical procedures

Protein was estimated by the Folin reaction [25] or by absorption at 280 nm. Amino acid analysis was carried out on a Durrum analyzer, Model D-500 after hydrolysis for 24 h at 110 °C in tubes placed in a sealed glass bomb in the presence of 6 M HCl and under N_2 atmosphere. Carbohydrates were derivatized to trimethylsilyl methyl glycosides [26] and analyzed by gas chromatography on a Hewlett-Packard model 5710 A gas chromatograph. The sugars were identified by their retention time as compared to known standards. Inositol (10–20 μg) was added as an internal standard. Electrophoresis in sodium dodecyl sulfate-polyacrylamide gels was done according to Fairbanks et al. [27]. Radioactivity was estimated in a Beckman scintillation spectrometer Model LS230 using a toluene-based scintillation liquid (Liquifluor, NEN).

RESULTS

Binding of ^{125}I-sialoglycoprotein from human erythrocyte membranes to wheat germ agglutinin-Sepharose 4B

The experiments summarized in Fig. 1 showed that the conjugated wheat germ agglutinin binds large amounts (90–95 %) of the added sialoglycoprotein isolated from human erythrocyte membranes. Approximately 180 μg of glycoprotein could be bound to 360 μg of conjugated wheat germ agglutinin. However, it is important to note that this degree of binding and elution of the labeled glycoprotein was obtained

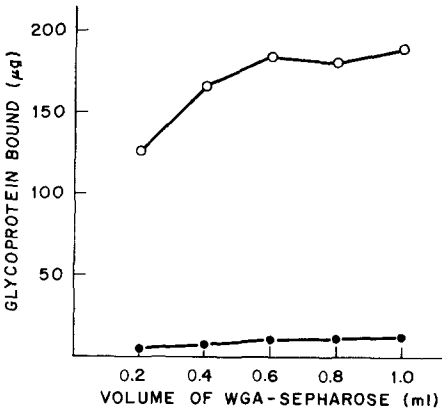

Fig. 1. Binding of isolated human red blood cell glycoprotein to wheat germ agglutinin (WGA)-Sepharose 4B. A 50 % suspension (625 μg wheat germ agglutinin/ml) of beads was prepared in buffer A with or without 0.25 M *N*-acetylglucosamine (GlcNAc). Different amounts of beads were sampled and incubated with 200 mg isolated human red blood cell ^{125}I-labeled glycoprotein for 30 min at room temperature. The beads were washed with phosphate-buffered solution (3 × 6 ml) and the bound radioactivity was estimated in a gamma counter: (○–○) binding in the absence of GlcNAc; (●–●) binding in the presence of GlcNAc.

TABLE I

THE EFFECT OF SIMPLE SUGARS ON THE BINDING OF ^{125}I-LABELED HUMAN ERYTHROCYTE GLYCOPROTEIN TO WHEAT GERM AGGLUTININ-SEPHAROSE

Sugar added	Concentration (M)	Binding (% inhibiton)
None	–	0
N-Acetyl-D-glucosamine	0.1	78
	0.06	59
N-Acetyl-D-galactosamine	0.1	10
	0.05	5
D-Glucose	0.1	7
	0.05	1
D-Mannose	0.1	10
	0.05	7
D-Galactose	0.1	3
	0.05	10
L-Fucose	0.1	0
	0.05	7
α-Methyl-D-glucoside	0.1	0
	0.05	0
α-Methyl-D-mannoside	0.1	4
	0.05	4

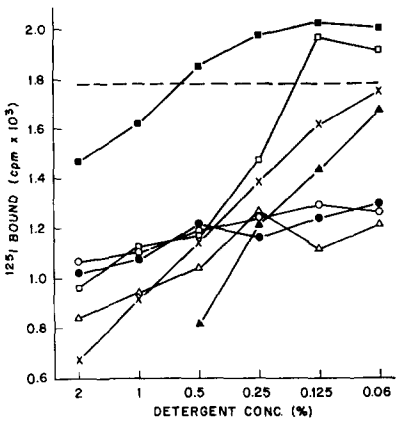

Fig. 2. Binding of the isolated human red blood cell glycoprotein to wheat germ agglutinin-Sepharose 4B conjugate in the presence of detergents and dissociating agents. The binding was performed as described in Fig. 1, except that 0.5 ml beads and 50 μg glycoprotein were used. Binding in the presence of lithium diiodosalicylate ■, dodecyltrimethylammonium bromide □, sodium deoxycholate ×, sodium dodecyl sulfate-labeled ▲, Triton X-100 ●, Lubrol PX ○, hexadecyltrimethylammonium bromide △, phosphate-buffered saline (- - -).

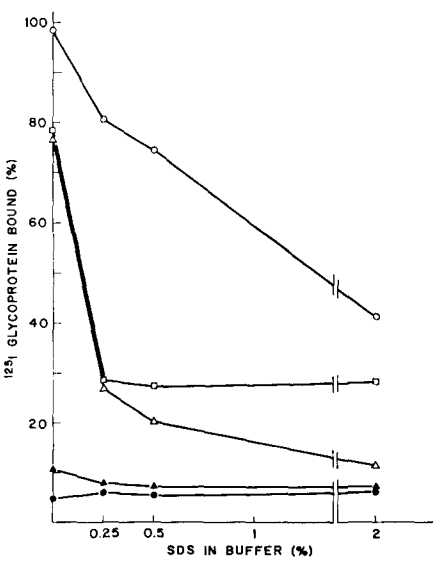

Fig. 3. Binding of ^{125}I-labeled human red blood cell glycoprotein to different lectin Sepharose 4B conjugates in the presence of sodium dodecyl sulfate (SDS). The binding was performed as described in Fig. 1, but 1 ml of conjugate and 100 μg glycoprotein were used. The lectins conjugated were wheat germ agglutinin (○), *Ricinus communis* agglutinin (□), erythroagglutinating phytohemagglutinin (△), leucoagglutinating phytohemagglutinin (▲) and concanavalin A (●).

only for glycoprotein preparations iodinated enzymatically by the lactoperoxidase procedure, while only 50–60 % binding was obtained for glycoprotein iodinated by the Chloramine T method [28]. The binding of the sialoglycoprotein could be inhibited completely by the monosaccharide *N*-acetyl-D-glucosamine (Fig. 1); and more than 90 % of the bound sialoglycoprotein could be eluted from the wheat germ agglutinin-Sepharose beads with 0.1 M *N*-acetyl-D-glucosamine. Other monosaccharides were found to have little effect on the binding (Table I).

Binding of sialoglycopeptides to lectin-Sepharose 4B in detergents

To be useful for the purification of membrane glycoproteins, the lectin conjugates must retain their specific affinities under conditions used for the dissociation of membrane components. We tested the effects of detergents and similar reagents commonly used for dissolving membrane proteins on the binding to the wheat germ agglutinin-Sepharose conjugates. The results are summarized in Fig. 2. Most of the detergents tested decreased the binding of the sialoglycopeptides, but for some, binding was comparable to the control at low detergent concentrations (0.05 %). Two groups of detergents can be distinguished by their effects on the binding affinity. One group which included the nonionic detergents Lubrol and Triton X-100 and the cationic detergent hexadecyltrimethylammonium bromide, affect binding almost independently of the detergent concentration. The second group, including lithium diiodosalicylate, deoxycholate, the anionic detergent sodium dodecyl sulfate and the cationic detergent dodecyltrimethylammonium bromide, caused a marked decrease in binding capacity at higher detergent concentrations. However, only sodium dodecyl sulfate was able to keep membrane proteins in solution at a detergent concentration that did not affect the binding capacity of the conjugated wheat germ agglutinin. Similar results were obtained when the binding of desialated glycophorin to wheat germ agglutinin-Sepharose was analyzed (data not shown). More than 95 % of the counts bound in sodium dodecyl sulfate could be eluted by a single wash in buffer containing 0.1 M *N*-acetylglucosamine. Since other lectins including erythroagglutinating phytohemagglutinin and *Ricinus communis* agglutinin are also able to bind the sialoglycopeptides, we compared their activities in sodium dodecyl sulfate and found that, unlike wheat germ agglutinin, concentrations of sodium dodecyl sulfate of 0.25 % or greater markedly inhibited the binding of glycophorin to these ligands (Fig. 3). Concanavalin A and leukoagglutinating phytohemagglutinin did not bind significant amounts of glycophorin, regardless of the sodium dodecyl sulfate concentration.

Isolation of glycophorin A by affinity chromatography on wheat germ agglutinin-Sepharose in sodium dodecyl sulfate

Human erythrocyte membranes were completely solubilized in 0.05 % sodium dodecyl sulfate (a concentration of detergent which affected binding of the sialoglycoproteins to the wheat germ agglutinin-Sepharose only slightly (see Fig. 2)), and when the solution of membrane components was applied to a wheat germ agglutinin-Sepharose column, equilibrated at the same concentration of detergent, almost all of the protein was found in the non-bound fraction. Only a small amount of protein was eluted with 0.1 M *N*-acetyl-D-glucosamine (Fig. 4) and this proved to be the major sialoglycoprotein. When intact cells had been labeled according to Blumenfeld et al. [21], a procedure which introduces ^{3}H exclusively into the sialic acids of the sialoglyco-

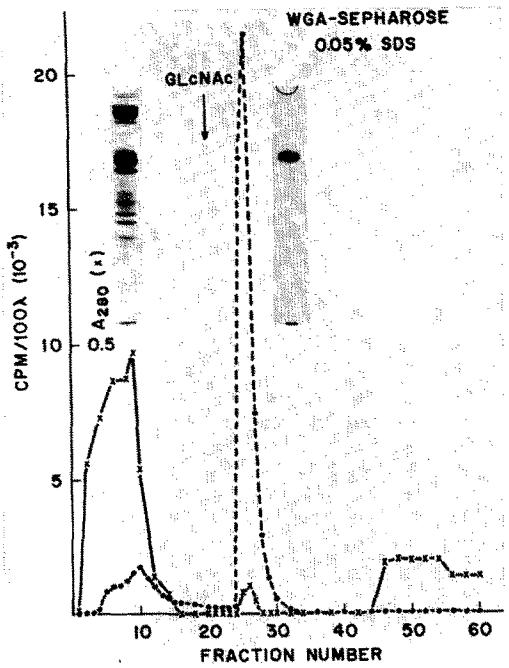

Fig. 4. Isolation of human red blood cell glycoprotein from red cell ghosts by affinity chromatography on wheat germ agglutinin-Sepharose 4B. Intact red blood cells were labeled with NaB^3H_4 and ghosts were prepared. Ghosts (30 mg protein) were solubilized in 1 % sodium dodecyl sulfate in 0.25 M NaCl in 0.015 M sodium phosphate, pH 7.2, (buffer B), and after centrifugation the supernatant was diluted to 0.05 % sodium dodecyl sulfate with buffer B, and was loaded on a column (1.2 × 7 cm) packed with wheat germ agglutinin conjugate. Elution of the bound material was done with 0.1 M *N*-acetyl-D-glucosamine (GlcNAc). (×–×) indicates absorption at 280 nm, (●–●) indicates ^3H counts. Inserted are sodium dodecyl sulfate gels stained with Coomassie blue of the non-bound material (left), which did not stain with periodic Schiff's reagent, and the eluted material (right), which gave identical patterns after staining by either method.

proteins, almost all of the counts were recovered in the eluted fraction (Fig. 4). Thus only the sialoglycoproteins are bound and elutable under these conditions. The eluate did not contain detectable free wheat germ agglutinin if the beads were prewashed with 2 % sodium dodecyl sulfate and equilibrated in 0.05 % sodium dodecyl sulfate in buffer B before application of the sample, and after elution, no radioactivity was retained with the beads, using ^3H- or ^{125}I-labeled ghosts, suggesting that trapping or nonspecific binding of membrane proteins is not a potential problem. The concentration of salt in the buffer used was found to be of great importance, since buffers of low ionic strength (5–10 mM) resulted in significantly more binding of protein to the beads. Under these conditions the eluted fraction was only slightly enriched for sialoglycoproteins and contained other membrane polypeptides, predominantly band 3 [27] indicating nonspecific binding or aggregation of these proteins.

Polyacrylamide electrophoresis in sodium dodecyl sulfate clearly showed that only the major sialoglycoprotein (glycophorin A) was found in the eluted fraction, when staining was done with Coomassie blue and periodic acid/Schiff's reagent (Figs. 5 and 6). All the Coomassie blue-staining polypeptides, seen in the original protein

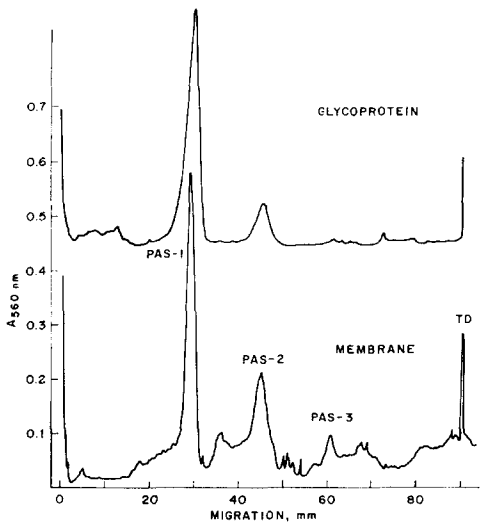

Fig. 5. Sodium dodecyl sulfate gel electrophoresis of peptides isolated by wheat germ agglutinin-affinity chromatography (top) in comparison to total membrane proteins (bottom). The gels were scanned at 560 nm after periodic acid Schiff's reagent.

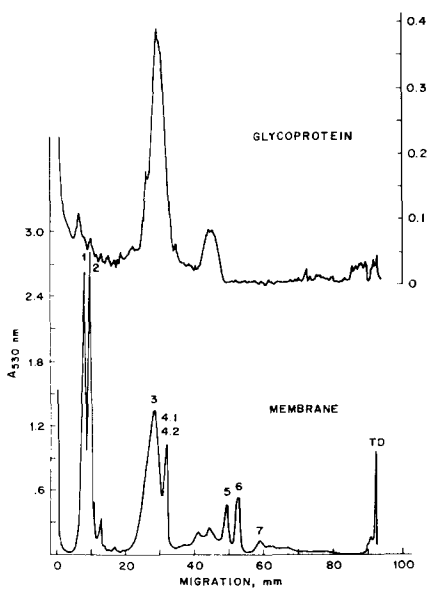

Fig. 6. Sodium dodecyl sulfate gel electrophoresis of peptides isolated by wheat germ agglutinin-affinity chromatography (top) in comparison to total membrane proteins (bottom). The gels were scanned at 550 nm after Coomassie blue staining.

TABLE II

AMINO ACID AND CARBOHYDRATE COMPOSITION OF GLYCOPHORIN A, PURIFIED BY WHEAT GERM AGGLUTININ-AFFINITY CHROMATOGRAPHY*

Asp	8.9	Carbohydrate	%
Thr**	13.1		
Ser**	14.5		
Glu	14.8		
Pro	10.6		
Gly	7.2	Fuc	1.5
Ala	7.9	Man	4.6
Cys***	–	Gal	19.7
Val	11.6	Glc	0.7
Met**	1.6	GalNAc	18.1
Ile	10.3	GlcNAc	10.6
Leu	7.8	Sialic Acid	44.7
Tyr	3.0		
Phe	2.3		
His	5.4		
Lys	5.4		
Arg	6.0		
Trp†	–		

* Amino acid composition calculated for 131 amino acid residues [30]. Carbohydrate composition in % of total.

** Values are uncorrected for losses during hydrolysis.

*** Cysteine determined as cysteic acid after performic acid oxidation [33].

† Tryptophan was determined spectrofluorometrically and after hydrolysis with *p*-toluene sulfonic acid [34]. The dash denotes less than 0.1.

mixture, could be recovered in the non-bound fraction. The absence of a minor sialoglycopeptide, commonly designated PAS-3 [27], and more recently designated glycophorin B [29] in both the non-bound and the eluted fraction, indicated that this sialoglycopeptide had been lost during washing of the column. Under optimal conditions, 25–30 mg (dry weight) of glycophorin A were obtained from 1 g of lyophilized erythrocyte membrane or one unit of blood. The carbohydrate and amino acid content was 59.9 and 40.1 %, respectively, which is consistent with values reported for this sialoglycoprotein. The amino acid composition was found to be very similar or identical to that reported for glycophorin A (Table II) [29, 30]. We have also found that the sialoglycopeptides purified with the wheat germ agglutinin-Sepharose system are identical in terms of sodium dodecyl sulfate gel patterns and amino acid and carbohydrate composition, regardless of whether the starting materials were intact red blood cell ghost membranes, lithium diiodosalicylate-treated membrane fragments [31] or the soluble glycoprotein fraction prepared by the lithium diiodosalicylate-phenol procedure [24].

DISCUSSION

The results presented here demonstrate that affinity chromatography is a potentially useful technique for the quantitative isolation of membrane glycoproteins. It is also clear that membrane proteins present special problems which must be solved

before this approach can be used more widely. Chief among these is the need to carry out the reactions in media capable of dissociating integral proteins from membrane lipids and maintaining the individual proteins in the dissociated state. We have explored the capacities of different detergents to both solubilize the glycoproteins of the human red cell membrane and allow specific association of their oligosaccharide moieties with conjugates of purified lectins.

Using the known interactions between wheat germ agglutinin and glycophorin A, the major sialoglycoprotein of the human red cell membrane, as a model system, we have found that low concentrations of sodium dodecyl sulfate in the presence of high salt will allow the specific binding and elution of soluble glycoprotein from conjugates of wheat germ agglutinin-Sepharose. The procedure we have developed is rapid, which lessens the likelihood of degradation, is inexpensive and results in the quantitative recovery of appropriate membrane glycoproteins free of detectable contaminants.

It was fortuitous that sodium dodecyl sulfate-wheat germ proved to be the most effective detergent-lectin combination for the isolation of glycophorin, since sodium dodecyl sulfate is generally considered to be one of the most effective agents to solubilize membranes, and wheat germ agglutinin is probably one of the most durable of the lectins. None of the other detergents were found to be very useful in this system, mostly because the membrane components were not kept in soluble form (as judged by phase contrast microscopy) at detergent concentrations that did not affect binding to any great extent. The resistance of wheat germ agglutinin to denaturation or inactivation by sodium dodecyl sulfate may be due to large numbers of disulfide bonds reputed to hold the polypeptide together. Buffers containing 2% sodium dodecyl sulfate were used to wash the wheat germ agglutinin-Sepharose beads without ill effects, but the binding activity of the wheat germ agglutinin was lost if reducing agents were included with the detergent.

Conjugation of purified wheat germ agglutinin to Sepharose beads was achieved with significantly lower amounts of cyanogen bromide and for much shorter times than was originally suggested for optimal coupling [20]. We devised this modification in the hope that only the most peripheral portions of the Sepharose beads would be activated, so that the bulk of the attached ligand would be accessible to the glycoprotein-detergent complexes, which might not be small enough to diffuse into the interior of the beads. We have no way of knowing whether this accounts for the effectiveness of our conjugates. Another important feature of this method is the use of sodium dodecyl sulfate in relatively high concentration (0.15–0.25 M range) salt solutions. When lower ionic strength buffers are used aggregation or nonspecific adsorption occurs. The presence of salt may modify the effects of sodium dodecyl sulfate on both the ligand and the dissociated glycoproteins, possibly through some effects on the micellar structure of the detergent. Ion exchange properties of cyanogen bromide activated Sepharose derivatives have been reported [37]. Despite the use of glycine to ensure complete blocking of active groups [38], which introduces free carboxyl groups, such effects probably cannot account for the nonspecific adsorption observed at low ionic strength.

This method was initially used to isolate glycophorin A from erythrocyte membranes, principally because this glycoprotein is a well characterized molecule and suitable for assessing both the quantitative and qualitative capacities of the procedure.

Glycophorin isolated by this procedure was indistinguishable chemically and comparable in amount to that obtained by the lithium diiodosalicylate-phenol method [24] and 5–10 times greater in amount than that recovered by other procedures [32]. We have also used this method as a critical step in the isolation of another major integral glycoprotein of the red cell membrane [31] and studies now in progress indicate that this may be a promising way to isolate glycoproteins from human tumor tissue (Leung, M. M., Kahane, I. and Marchesi, V. T., unpublished observations).

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