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GLYCOPROTEIN CHARACTERISTICS OF THE SODIUM CHANNEL SAXITOXIN-BINDING COMPONENT FROM MAMMALIAN SARCOLEMMA

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The saxitoxin-binding component of the excitable membrane sodium channel exhibits glycoprotein characteristics as evidenced by its specific interaction with various agarose-immobilized lectins. The detergent-solubilized saxitoxin-binding component interacts quantitatively with immobilized wheat germ agglutinin and concanavalin A and fractionally with immobilized *Lens culinaris* hemagglutinin and *Ricinus communis* agglutinin. These lectins preferentially bind *N*-acetylglucosamine and sialic acid (wheat germ agglutinin), mannose (concanavalin A and *Lens cunilaris*) and galactose (*Ricinus communis*). Removal of terminal sialic acid residues by neuraminidase markedly decreases binding to immobilized wheat germ agglutinin but uncovers sites capable of interacting with lectins specific for galactose and *N*-acetylgalactosamine. β-*N*-acetylglucosaminidase, an exoglycosidase, has no effect on the binding of the channel protein to wheat germ agglutinin. Similarly, phospholipase C has no effect on binding of the solubilized toxin binding component to this lectin. Neither wheat germ agglutinin nor concanavalin A free in solution alters the number of toxin binding sites or their affinity for toxin. The sodium channel saxitoxin-binding component appears to be a glycoprotein containing terminal sialic acid residues and internal mannose, galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine residues. The toxin binding site is spatially separated from the binding sites for the lectins studied. The effect of these sugar moieties must be considered when evaluating the biophysical parameters of the sodium channel.

Introduction

In excitable membranes, action potentials are produced by sequential time- and voltage-dependent changes in membrane conductance to sodium and potassium ions [1]. The rapid depolarizing phase of the action potential is thought to be associated with movement of sodium ions through protein pores that traverse the membrane [2]; these pores are commonly referred to as sodium channels. While much electrophysiologic information on these channels has been amassed, biochemical and molecular studies have begun only recently.

The excitable membrane sodium channel can be quantitated in intact membranes and after detergent solubilization by the specific binding of the low molecular weight neurotoxins, saxitoxin and tetrodotoxin [3]. Studies in a variety of excitable membranes have shown that toxin binding is specific (one toxin molecule per channel) and reversible, with K_d values in the nanomolar range [4,5]. These toxins bind to a site accessible only on the outside of the cell and cause a specific block of voltage-dependent sodium currents [4].

Recently, considerable progress has been made toward the purification of the sodium channel

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Abbreviations: SBC, saxitoxin binding component of the sodium channel; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine.

protein from eel electroplax [6] and from rat sarcolemma [7] using the binding of ^{3}H-labeled saxitoxin or tetrodotoxin to identify the channel toxin binding component following solubilization. In rat sarcolemma, part of the purification scheme depended on the apparent glycoprotein nature of the sodium channel saxitoxin-binding component (SBC) as evidenced by its specific interaction with wheat germ agglutinin [7]. The purified sarcolemmal SBC retained the toxin binding characteristics of the in situ sodium channel.

In the present paper, the glycoprotein nature of the SBC is further characterized by studying its interation with lectins specific for cell membrane saccharides in addition to those which interact with wheat germ agglutinin and by studying the effects of various enzymes on the lectin binding properties of the sodium channel SBC.

Experimental procedures

Materials. Sepharose-immobilized wheat germ agglutinin, concanavalin A, *Lens culinaris*, and *Helix pomatia* lectins were obtained from Pharmacia, Piscataway, NJ. Agarose-bound soybean agglutinin, *Ulex europeus*, *Arachis hypogaea*, *Ricinus communis* and *Bandeiraea simplicifolia* lectins were obtained from Vector Laboratories, Burlingame, CA. Agarose-immobilized *Abrus precatorius* and *Dolichos biflorus* lectins were obtained from PL Biochemicals, Milwaukee, WI. All of the sugars used, the purified phosphatidylcholine (PC), neuriminidase type V from *Clostridium perfringens* (EC 3.2.1.18), phospholipase C type X from *C. perfringens* (EC 3.1.4.3), β-*N*-acetylglucosaminidase from Jack beans (EC 3.2.1.30), lyophilized wheat germ agglutinin and concanavalin A, and choline chloride were obtained from Sigma Chemical Company, St. Louis, MO. Nonidet P-40 was the product of BDH Chemical, Ltd., Poole, U.K. Unlabelled tetrodotoxin was obtained from Calbiochem, La Jolla, CA. Unlabelled saxitoxin was the generous gift of Dr. E. Shantz of the University of Wisconsin.

Preparation and solubilization of purified sarcolemmal membranes. Membranes were prepared from rat hind limb muscle by a modification of the LiBr extraction procedure of Festoff and Engel [8] as reported by Barchi et al. [9]. Purified sarcolemma was suspended in Tris buffer (5 mM, pH 7.4 at 4°C) and stored frozen at −20°C prior to use. Sarcolemma was solubilized by ten strokes in a teflon-glass homogenizer in a solution of 2 mg/ml sarcolemmal membrane, 50 mM KH_2PO_4, (pH 7.4, 0°C), 0.5 mM $CaCl_2$ and 1% Nonidet P-40. The solubilized material was centrifuged at 100 000 × *g* for 30 min to yield the supernatant fraction used for all binding studies below. All subsequent buffers used to dilute the solubilized material, to elute columns and to run binding assays contained 0.1% Nonidet P-40 supplemented with phosphatidylcholine in a 5 : 1 molar ratio of detergent to phospholipid. All operations were carried out at 0–5°C.

Saxitoxin binding assays. Saxitoxin was tritiated, purified, and assayed for biological activity and radiopurity as previously described [10]. Binding of ^{3}H-labeled saxitoxin to purified membrane fractions was determined by a rapid filtration assay using micropore glass-fiber filters [10]. Binding to solubilized fractions was determined by a modification of the micro-column assay of Levinson et al. [11] as previously described [3]. Activity was corrected (when assaying column fractions) for non-specific binding, column background, and counting efficiency and is reported where indicated as pmole of saxitoxin binding per mg of protein. A fluorescamine assay was used for protein determinations [12].

Lectin column assays. In general, 1-ml lectin columns were prepared in 3-ml plastic syringe barrels and equilibrated with 10 ml of buffer (50 mM KH_2PO_4, pH 7.4 at 0°C, 0.1% Nonidet P-40/phosphatidylcholine (5 : 1 molar ratio), and 200 mM choline chloride). Varying amounts of previously solubilized sarcolemmal membranes were applied to the columns in a minimal volume. The gel was washed with 8 ml of buffer and eluted with the appropriate sugar (Table I). Columns were run at 0.25 ml/min; 1-ml fractions were collected.

Enzymatic treatment of purified sacrolemmal membranes. Purified sarcolemmal membranes were incubated at 2 mg/ml and 25°C in 50 mM KH_2PO_4, pH 7.0, with one unit per ml of neuraminidase. Aliquotes were removed periodically for determination of released sialic acid by the method of Warren [13,14]. Neuraminidase action was terminated by diluting the membranes with ice-cold phosphate buffer and spinning at 100 000 × *g* to pellet the mem-

TABLE I

CHARACTERISTICS OF LECTINS USED IN THIS STUDY

Lectins [a]	Source	Elution sugar	Oligosaccharide specificity
D-Mannose			
Concanavalin A	Jack bean	α-Methyl-D-mannoside	Dextrans, mannans,
Lens culinaris	Lentil bean	α-Methyl-D-mannoside	D-fructous, internal 2-*O*-substituted D-mannopyronosyl residues
N-Acetylglucosamine			
Wheat germ	*Triticum vulgaris*	GlcNAc	*N,N',N''*-Triacetyl chitotriose [b]
N-Acetyl-galactosamine			
Helix pomatia	Snail's albumen gland	GalNAc	A-active trisaccharide [c]
Soybean	*Glycine max*	GalNAc	A-active trisaccharide
Dolichos biflorus	Horse gram	GalNAc	A-active trisaccharide
D-Galactose			
Ricinus communis 1(RCA_{120})	Castor bean	Lactose	Lactose
Abrus precatorius	Jequirity bean	D-(+)-Galactose	Lactose
Arachis hypogaea	Peanut	D-(+)-Galactose	β-D-Galactosyl(1 → 3)-D-GalNAc
Bandeiraea simplicifolia 1	*B.s.* seed	D-(+)-Galactose	2-*O*αD-Galactopyranosyl-D-glucose
α-L-Fucose			
Ulex europeus 1	Gorse or furze seed	α-L-Fucose	2'-*O*-L-Fucosyllactose

[a] Lectins arranged by monosaccharide specificity.
[b] β-(1 → 4)-linked $(GlcNAc)_3$.
[c] α-D-GalNAc(1 → 3)β-D-Gal(1 → 3)GlcNAc.

branes. The membranes were resuspended in ice-cold 5 mM Tris, pH 7.4 and solubilized as previously described. Binding to lectin columns was then quantitated.

For studies with β-*N*-acetylglucosaminidase, 5 units of enzyme per ml were used at a pH of 6.5. Phospholipase studies were done with one unit of enzyme per ml at pH 6.5.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gels with a 7 to 20% linear acrylamide gradient were run at 4 V/cm for 18 h following the method of Laemmli [15]. Gels were stained with Coomassie Brilliant Blue.

Results

Binding of the solubilized sodium channel SBC to immobilized lectins

The binding of the sodium channel saxitoxin-binding component (SBC) to lectins specific for each of the commonly found cell surface saccharides was examined (Table II). In each case it was necessary to determine the maximum binding capacity of the gel in order to distinguish limited binding due to gel saturation from fractional binding attributable to a subgroup of the applied SBC possessing sugar moieties accessible for interaction with the particular immobilized lectin. In all cases, the data reported is the average of three or more column runs, each involving a different protein-to-gel ratio.

Wheat germ agglutinin

Of all the lectins studied, wheat germ agglutinin immobilized to Sepharose was the only one found to interact quantitatively and reversibly with the solubilized SBC. Binding was highly selective: while less than 5% of the total solubilized sarcolemmal proteins was retained by the immobilized wheat germ agglutinin, virtually all (more than 95%) of the saxitoxin-binding activity was adsorbed specifically (Fig. 1). Binding could be inhibited by the inclusion of 10 mM *N*-acetylglycosamine (GlcNAc) in all buffers but was unaffected by increasing the ionic strength up to 800 mM choline chloride. Elution occurred specifically

TABLE II

LECTIN BINDING CHARACTERISTICS OF THE SOLUBILIZED SODIUM CHANNEL SBC

The experimental data in this table is the average of at least three separate determinations. See Table I for explanation of the abbreviations used.

Lectins	% of SBC Bound		Measured maximum binding capacity (pmol/mg of gel)	Theoretical binding site density (nmol/mg of gel)
	Native SBC	Neuraminidase treated SBC		
D-Mannose (Glucose)				
Con A	90	90	4.0	385
LcH	30	32	0.9	77
N-Acetylglucosamine				
WGA	>95	37	2.0	555
N-Acetylgalactosamine				
HPL	variable	23	0.3	76
SBA	0	9	0.2	66
DBL	0	30	0.4	67
D-Galactose				
RCA_{120}	25	58	0.9	67
APL	0	29	0.4	15
AHA	0	8	0.2	82
BSL-1	0	60	0.6	140
α-L-Fucose				
UEA	0	0	0	100

and reproducibly at 7–8 mM GlcNAc with recoveries often exceeding 90%. The wheat germ agglutinin-Sepharose column was capable of adsorbing dilute solubilized SBC from a large volume of detergent solution at high ionic strength (400 mM choline chloride) and of allowing subsequent elution in a small volume, thereby providing enrichment and concentration in a single step. Enhancement of specific saxitoxin-binding activity averaged 15- to 20-fold with crude solubilized sarcolemma and close to 20-fold with SBC partially prepurified on a guanidinium ion exchange column [7]. Maintaining a high ionic strength minimized non-specific binding to the gel, although it was also observed to decrease the gel's apparent binding capacity. Moderate overloading of the column or running the column in the presence of a weakly competing sugar such as glucose resulted in a more selective retention of specific saxitoxin-binding activity.

To assess the possibility that wheat germ agglutinin might sterically or conformationally affect toxin binding to the sodium channel SBC, equilibrium binding curves at saxitoxin concentrations between 0.1 and $10 \cdot 10^{-9}$ M were constructed in the presence of 100 and 500 μg/ml free wheat germ agglutinin in solution with sarcolemmal membranes. No change in either saxitoxin-binding affinity (K_d) or total number of binding sites (B_{max}) was observed compared to control binding curves done in the absence of free lectin. Thus the lectin binding site appears to be spatially separated from the binding site for saxitoxin and tetrodotoxin. SDS-polyacrylamide gel electrophoresis on 7% to 20% linear gradient gels was performed on samples of solubilized sarcolemma, protein not bound to immobilized wheat germ agglutinin, and protein specifically eluted from this lectin by GlcNAc during a column run such as that in Fig. 1. The gel profile of the unretained protein closely resembled that of the solubilized sarcolemma with the notable absence of a band at 140 000 (Fig. 2). The banding pattern of the bound protein differed from both the initial sarcolemma and the unbound protein. Four prominent bands are seen between 40 000 and 60 000 daltons, and one each at 66 000 and 78 000 daltons

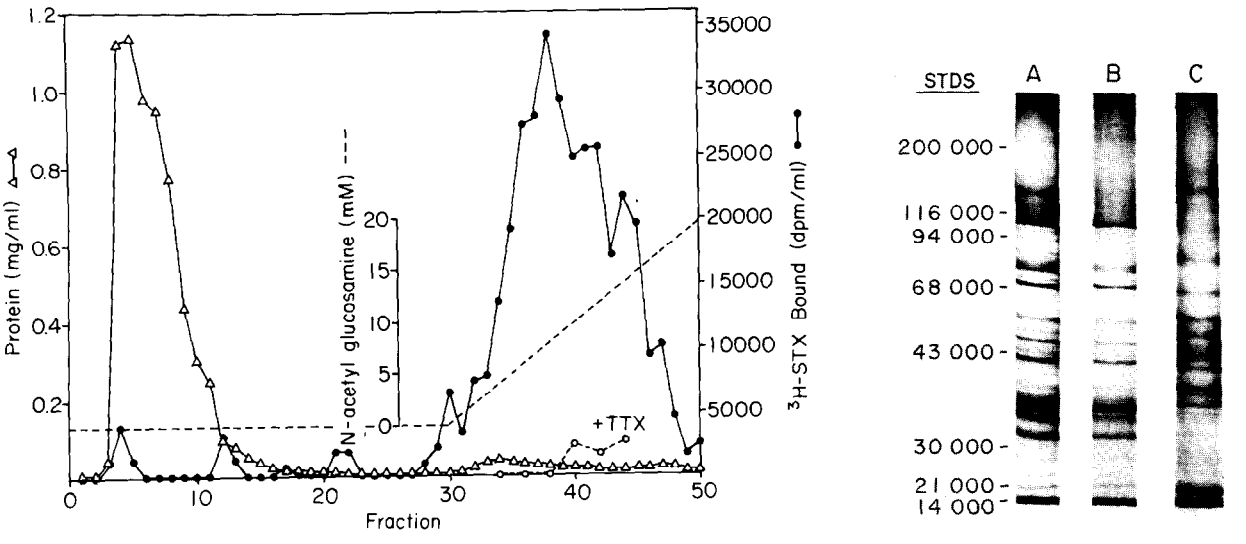

Fig. 1. Affinity chromatography of solubilized sarcolemma on a column containing wheat germ agglutinin immobilized to Sepharose 6MB beads. The column (1.6 X 12 cm) was equilibrated in 0.1% Nonidet P-40/phosphatidylcholine, 400 mM choline chloride, and 50 mM KH_2PO_4 (pH 7.4). Sarcolemma was solubilized at 2 mg of protein per ml with 1% Nonidet P-40, and 6.0 mg was applied to the column in running buffer at 0.5 ml/min. The column was eluted with a 0–20 mM gradient of *N*-acetylglucosamine at the same flow rate; 5-ml fractions were collected. △———△, Total protein (mg/ml); •———• total ^{3}H-labeled saxitonin bound (dpm/ml); ○———○, non-specific binding (dpm/ml).

Fig. 2. Gradient (7–20%) sodium dodecyl sulfate/polyacrylamide gel electrophoresis of (A) solubilized sarcolemma, (B) fraction of solubilized sarcolemma not retained by wheat germ agglutinin-Sepharose 6MB. and (C) fraction bound to and eluted from wheat germ agglutinin-Sepharose 6MB. Approx. 20 μg of protein was applied to each lane.

which are only trace components in the crude sarcolemma. The material banding in sarcolemma at 140 000 daltons but absent in the unbound proteins is recovered in the specifically bound fraction.

The experiments reported above were carried out with solubilized sarcolemma in which the sodium channel toxin binding component represents only a minor fraction of the total membrane protein. Subsequent experiments with the SBC purified from sarcolemma as previously reported [7] yielded identical results, confirming that the lectin binding properties are a characteristic of this channel protein and not attributable to the presence of other membrane proteins.

Concanavalin A

Concanavalin A immobilized to Sepharose beads (Con A-Sepharose) adsorbed 60% of the solubilized sarcolemmal protein and 90% of the sodium channel SBC. Only partial recovery of the SBC was possible by elution with sugar. About 20% of the adsorbed SBC activity could be eluted with 800 mM α-methyl-D-mannoside (or glucoside), while 10% was eluted with 20% ethylene glycol and 1% with 100 mM borate buffer alone (pH 7.4 at 0°C). As with wheat germ agglutinin, concanavalin A free in solution did not affect either the binding affinity or the total number of specific saxitoxin-binding sites when assayed at 100 and 500 μg lectin/ml.

In order to distinguish inactivation of toxin binding activity due to Con A-Sepharose–SBC interaction from irreversible high affinity binding to the gel, the ability of the solubilized channel protein adsorbed to Con A-Sepharose to specifically bind saxitoxin was quantitated. Con A-Sepharose was mixed with solubilized sarcolemmal protein in a batch process and then washed with buffer. Solubilized SBC bound to the immobilized lectin was assayed for total and non-specific binding of ^{3}H-labeled saxitoxin following

incubation with labelled toxin by trapping aliquots of the gel on glass fiber filters. Preservation of specific binding was quantitative, indicating that the sodium channel SBC bound to Con A-Sepharose retained its ability to bind saxitoxin specifically.

Other lectins

Lens culinaris hemagglutinin qualitatively resembles concanavalin A in its saccharide-binding properties. Its affinity for mannose and glucose is lower, however, often yielding better selectivity and recovery of bound protein when compared with immobilized concanavalin A [16,17]. Immobilized *Lens culinaris* lectin bound 30% of the applied sodium channel SBC but only 10% of the solubilized sarcolemmal proteins. Specific binding decreased by half in the presence of 250 mM choline chloride, indicating either significant ion chromatographic or weak affinity properties to be present with this gel. Retained saxitoxin-binding activity was recovered with 60 to 80% efficiency with 600 mM α-methyl-D-mannoside or -glucoside.

Of the four lectins studied with specificities for galactose, only *Ricinus communis* agglutinin interacted specifically with the solubilized sodium channel SBC (Table II). 25% of the applied SBC was bound by a column containing this immobilized lectin independent of ionic strength, and this fraction could be quantitatively eluted with 100 mM α-lactose.

No binding to the other lectins specific for D-galactose (*Abrus precatorius*, *Arachis hypogaea*, or *Bandieraea simplificolia*) could be demonstrated; recovery of specific activity in the wash after loading of the solubilized sarcolemma was quantitative. No binding could be demonstrated to L-fucose-specific *Ulex europeus* lectin.

Of the lectins specific for *N*-acetylgalactosamine (GalNAc), no specific binding could be demonstrated with *Glycine max* (soybean)- or *Dolichos biflorus* lectin immobilized to agarose while variable results were obtained with immobilized *Helix pomatia* lectin. Out of seven column runs with *Helix pomatia*-Sepharose, four showed no specific binding while three produced variable amounts of binding (26, 52 and 88%) but with identical elution patterns: gradient elution yielded two peaks at concentrations of 6 and 15 mM GalNAc. No conditions could be found which yielded reproducible results with this lectin.

Effect of enzymatic treatment on lectin binding

A series of enzymatic digestions were performed directed at confirming the presence of specific saccharide moieties on the channel and probing their structure. Biochemical manipulation of the sodium channel protein after removal from the membrane in solubilized form has been made feasible by the recent recognition of the role of phospholipids in stabilizing the channel protein [3,18] and evidence suggests that the sodium channel SBC exists in solution as a lipid-protein complex. Sarcolemmal membranes were extensively treated with phospholipase C (one unit/ml at 37°C, pH 6.5). A 25% reduction in specific binding of ^{3}H-labeled saxitoxin was observed, confirming the findings of Baumgold [19] but in disaggreement with those of Chacko [20]. Treatment of sarcolemma with phospholipase C, however, produced no effect on either binding or elution of the subsequently solubilized SBC from wheat germ agglutinin-Sepharose compared to control.

Wheat germ agglutinin binds to oligosaccharides containing internal and terminal GlcNAc as well as terminal sialic acid residues [21–27]. Since either or both of these saccharides could be present on the SBC, an attempt was made to distinguish between the two using *N*-acetylglucosaminidase and neuraminidase. No effect on specific saxitoxin binding to the SBC or on SBC binding by the immobilized lectin was observed following exposure of the channel to the glucosaminidase under common reaction conditions [28]. Activity of this terminal glycosidase with an artificial substrate (*p*-nitrophenyl-β-2-acetamido-2-deoxy-D-glycopyranoside) under the conditions used in this experiment was 209 nmoles of GlcNAc liberated per min per unit of enzyme.

The role of terminal sialic acid residues in binding to immobilized wheat germ agglutinin was examined by incubation of sarcolemma with 1 unit/ml of neuraminidase (pH 7.0 and 25°C). Release of sialic acid was monitored by the procedure of Warren [13,14]. After washing these membranes and solubilizing the SBC, specific binding to wheat germ agglutinin-Sepharose decreased from a control value of 95% at zero time to 37% at 10 h incubation (Fig. 3). No significant change in the total concentration of saxitoxin-binding sites in solution occurred under these or any subsequent incubation conditions.

Over the same time interval, a small decrease in

binding to the lectin was observed with the untreated control sarcolemma. This decreased binding ranged from 5 to 20% and varied with different sarcolemmal preparations. This could be the result of the action of different amounts of endogenous proteolytic enzymes or intrinsic sialidase activity in the different sarcolemmal preparations. In order to control for endogenous proteolysis, sarcolemmal membranes were incubated with neuraminidase for 10 h in the presence of a protease inhibitor cocktail (0.1 mM phenylmethylsulfonyl fluoride, 0.1 μl/ml leupeptin, 0.1 μg/ml pepstatin, 1 mM 1,10-phenanthrolene, and 1 mM iodoacetamide). A decrease in binding to immobilized wheat germ agglutinin comparable to control membranes was still observed, however.

Sarcolemma treated for 10 hours with neuraminidase was washed, pelleted, and solubilized, and the resultant SBC tested for binding to immobilized lectins specific for other common sugar groups, (see the 'Neuraminidase treated SBC' column, Table II). Binding to concanavalin A and *Lens cunilaris* lectin immobilized to sepharose was not affected by pretreatment with neuraminidase, but binding could now be demonstrated to immobilized lectins specific for D-galactose and GalNAc, lectins which had previously retained no SBC. No binding could be detected to α-L-fucose specific *Ulex europeus* lectin.

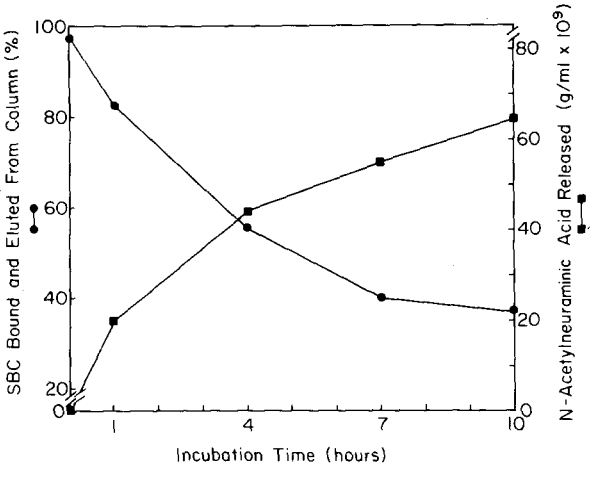

Fig. 3. Role of terminal sialic acid residues in binding to immobilized wheat germ agglutinin. A 2 mg/ml solution of sarcolemma membrane was incubated with 1 unit/ml of neuraminidase at pH 7.0 and 25°C. Aliquots were removed at various times and assayed for release of sialic acid (specifically, *N*-acetylneuraminic acid) by the procedure of Warren [13,14] and after solubilization, for binding to immobilized wheat germ agglutinin. ▪——▪, *N*-acetylneuraminic acid released during incubation, 10^{-9} g/ml; •——•, percentage of SBC bound and eluted from the wheat germ agglutinin column.

Discussion

The affinity of the solubilized sodium channel saxitoxin-binding component (SBC) for various sugar-specific lectins and the effect of neuramidase on its binding to these lectins support the hypothesis that this channel component is a sialoglycoprotein. The SBC binds, under various conditions, to lectins specific for sialic acid, GlcNAc, GalNAc, galactose, and mannose residues and, as such, has characteristics of both *N*-glycosidically and *O*-glycosidically linked glycopeptides and probably contains a mixture of both types.

The interaction of the SBC with immobilized wheat germ agglutinin is quantitative and specific, exhibiting each of the characteristics of a specific affinity interaction as described by Lowe and Dean [29]. Binding appears to be due to both terminal sialic acid residues and internal or possibly sterically 'hidden' terminal GlcNAc residues as evidenced by the studies with β-*N*-acetylglucosaminidase and neuraminidase, both exoglycosidases. The specific interaction of the SBC with wheat germ agglutinin-Sepharose allows significant enhancement of specific activity and has been used as a step in purification of the mammalian sodium channel from rat sarcolemma [7]. Preliminary experiments indicate that the specific interaction with immobilized wheat germ agglutinin can be used to advantage with sodium channel protein from other sources, including eel electroplax and mammalian synaptosomes.

Both specific and non-specific interaction with immobilized concanavalin A appear to be present since only 20% of the adsorbed SBC could be eluted from concanavalin A by mannose and nearly as much could be displaced by non-specific means (e.g., ethylene glycol and borate buffer). Binding to concanavalin A can occur not only by specific carbohydrate recognition but also by hydrophobic interaction between lectin and ligand [22,23,29]. Furthermore, polyelectrolytes and polysaccharides have been observed to

form strong ionic and hydrogen bonded non-specific interactions with concanavalin A [30]. Various proteins have been observed to interact through different combinations of these factors [31,32] and the solubilized SBC may fall into this group.

The variable fractional binding of the sialo- and asialo-sodium channel SBC to the other immobilized lectins suggests the presence of structural heterogeneity similar to that commonly found in the carbohydrate moieties of glycoproteins [21,22,33,34]. This heterogeneity may be due in part to artifacts introduced when glycosidases are released from broken cells during membrane isolation. However, even when these artifacts are protected against, an inherent microheterogeneity is still often observed due to either incomplete glycosylation or partial degradation of a properly glycosylated oligosaccharide during biosynthesis. Such heterogeneity has been demonstrated in the saccharide groups of the detergent solubilized acetylcholine receptor [35] and of acetylcholinesterase [36].

The binding sites of most lectins are thought to be complementary to carbohydrate moieties more complex than the simple sugars used for their classification (Table I). In general, higher affinities are observed for the binding of lectins to soluble (or solubilized) glycoproteins or cell surface carbohydrates than to simple sugars [22]. These very high affinities are believed to be caused by the multivalent interactions of the more complex oligosaccharides [37]. Furthermore, a certain amount of 'permissiveness' exists in the recognition by lectins of saccharides that are similar but distinct from the best saccharide inhibitors [22]. Hence, the assignment of specific sugar groups to a glycoprotein based on lectin binding studies alone is only tentative.

Confirmation of the presence of specific sugar groups is most easily made by structural studies involving sugar-specific glycosidases [38]. Our enzyme studies suggest that SBC binding to wheat germ agglutinin is in part mediated by terminal sialic acid residues. However, while β-*N*-acetylglucosaminidase has no effect on SBC binding to immobilized wheat germ agglutinin, it is still possible that binding to this lectin involves some contributions by terminal GlcNAc inaccessable to enzymatic cleavage. In neuraminidase-treated SBC, the decrease in binding to wheat germ agglutinin-Sepharose was balanced by the appearance of an approximately equivalent amount of binding to two immobilized lectins specific for terminal D-galactose, *Ricinus communis* and *Bandeiraea simplicifolia* lectins. This suggests that an α-D-linked galactose occurs penultimate to a terminal sialic acid, a finding common to other systems as well [39].

Sites specific for terminal GalNAc also became apparent after neuraminidase treatment. This implies either that a second terminal sialic acid residue was cleaved, that endogenous or contaminating glycosidases further pared down the now accessible saccharide chain ending in galactose, or that removal of steric hinderance by the cleavage of the bulky, highly charged terminal sialic acid residue rendered previously inaccessible GalNAc available for lectin binding. All three explanations appear possible. The marked variability of binding by the different lectins may be explained by the different affinities of the lectins for terminal GalNAc or may be due to 'permissiveness' as prevously described.

Since most intrinsic membrane glycoproteins contain sugar residues on those portions exposed on the external surface of the membrane, the sidedness of the sarcolemmal vesicles used for these enzyme studies is an important consideration. We have previously demonstrated that virtually all sarcolemmal vesicles are in fact oriented right-side out as evidenced by electron histochemical localization of lectin binding and by comparison of binding of ^{3}H-labeled saxitoxin before and after vesicle solubilization [9,40].

The above experiments were carried out in the presence of mixed detergent-phospholipid micelles composed of Nonidet P-40 and phosphatidylcholine. Although some detergents, in particular SDS, can either dissociate lectin molecules into their subunits or change lectin conformation into states of decreased or absent carbohydrate binding activity, this is probably not the case for Nonidet P-40. Work by Lotan and Nicolson [22] and Lotan et al. [41] indicates minimal if any effect on the binding properties of immobilized concanavalin A, wheat germ agglutinin, *Ricinus communis* and peanut and soybean agglutinins when used with 0.1% Nonidet P-40. The other lectins used in this study, however, have not been evaluated in this manner.

Glycoproteins exhibit anomalous behavior during gel filtration and gel electrophoresis due to their

increased extent of hydration [34]. The isoelectric and isoionic points can be affected significantly by the highly charged terminal sialic acid groups. Glycoproteins may migrate anomalously on SDS-acrylamide gels and stain poorly with the usual protein stains used in this system. The glycoprotein nature of the sodium channel SBC must be considered when evaluating the physical properties of the sodium channel SBC.

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References

1 Hodgkin, A.L. and Huxley, A.F. (1952) J. Physiol. (London) 117, 500–544
2 Hille, B. (1975) J. Gen. Physiol. 66, 535–560
3 Barchi, R.L. and Murphy, L.E. (1980) Biochim. Biophys. Acta 597, 391–398
4 Ritchie, J.M. and Rogart, R. (1977) Rev. Physiol. Biochem. Pharmacol. 79, 2–45
5 Weigele, J.B. and Barchi, R.L. (1978) FEBS Lett. 91, 310–314
6 Agnew, W., Levinson, S., Brabson, J. and Raftery, M. (1978) Proc. Natl. Acad. Sci. USA 75, 2606–2611
7 Barchi, R.L., Cohen, S.A. and Murphy, L.E. (1980) Proc. Natl. Acad. Sci. USA 77, 1306–1310
8 Festoff, B. and Engel, W.K. (1974) Proc. Natl. Acad. Sci. USA 71, 2435–2439
9 Barchi, R.L., Weigele, J.B., Chalikian, D.M. and Murphy, L.E. (1978) Biochim. Biophys. Acta 550, 59–76
10 Weigele, J.B. and Barchi, R.L. (1978) FEBS Lett. 91, 310–314
11 Levinson, S.R., Curatalo, C.T., Reed, G. and Raftery, M.A. (1979) Anal. Biochem. 99, 72–84
12 Bohlen, P., Stein, S., Dairman, W. and Udenfriend, S. (1973) Arch. Biochem. Biophys. 155, 213–220
13 Warren, L. (1959) J. Biol. Chem. 234, 1971–1975
14 Warren, L. (1973) Meth. Enzymol. 6, 463–465
15 Laemmli, U.K. (1970) Nature 222, 680–685
16 Young, N.M., Leon, M.A., Takahashi, T., Howard, I.K. and Sage, H.J. (1971) J. Biol. Chem. 246, 1596–1600
17 Hayman, M.J. and Crumpton, M.J. (1972) Biochem. Biophys. Res. Commun. 47, 923–930
18 Agnew, W.S. and Raftery, M.A. (1979) Biochemistry 18, 1912–1919
19 Baumgold, J. (1980) J. Neurochem. 34, 327–333
20 Chacko, G.K. (1979) J. Membrane Biol. 47, 285–301
21 Sharon, N. and Lis. H. (1975) Methods Membrane Biol. 3, 148–200
22 Lotan, R. and Nicholson, G.L. (1979) Biochim. Biophys. Acta 559, 329–376
23 Goldstein, I.J. and Hayes, C.E. (1978) Adv. Carbohydr. Chem. Biochem. 35, 127–340
24 Greenway, P.J. and LeVine, D. (1973) Nature New Biol. 241, 191–192
25 Adair, W.L. and Kornfeld, S. (1974) J. Biol. Chem. 249, 4696–4704
26 Vlodavsky, I. and Sachs, L. (1975) Exp. Cell. Res. 93, 111–119
27 Cruz, T.F. and Gurd, J.W. (1978) J. Biol. Chem. 253, 7314–7318
28 Li, S. and Li, Y. (1970) J. Biol. Chem. 245, 5153–5160
29 Lowe, C.R. and Dean, P.D.G. (1974) Affinity Chromatography, John Wiley and Sons, New York
30 Doyle, R.J., Woodside, E.E. and Fishel, C.W. (1968) Biochem. J. 106, 35–40
32 Davey, M.W., Huang, J.W., Sulkowski, E. and Carter, W.A. (1974) J. Biol. Chem. 249, 6354–6355
33 Marshall, P.N., (1972) Annu. Rev. Biochem. 41, 673–702
34 Spiro, R.G. (1973) Adv. Protein Chem. 27, 349–467
35 Salvaterra, P.M., Gurd, J.M. and Mahler, H.D. (1977) J. Neurochem. 29, 345–348
36 Gurd, J.W. (1976) J. Neurochem. 27, 1257–1259
37 Goldstein, I.J., Hammarstrom, S. and Sundblad, G. (1975) Biochim. Biophys. Acta 405, 53–61
38 Flowers, H.M. and Sharon, N. (1979) 48, 29–95
39 Codington, J.F., Linskey, K.B., Jeanloz, R.W., Irimura, T. and Osawa, T. (1975) Carbohydr. Res. 40, 171–182
40 Barchi, R., Bonilla, E. and Wong, M. (1977) Proc. Natl. Acad. Sci. USA 74, 34–38
41 Lotan, R., Beattie, G., Hubbell, W. and Nicolson, G.L. (1977) Biochemistry 16, 1787–1794