A Model System for Studies of Specific Membrane Interactions†

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ABSTRACT: We have developed a model system to study membrane interactions mediated by specific ligand–receptor binding. The acetylcholine receptor and α-bungarotoxin system has been chosen for this purpose. Purified α-bungarotoxin was reacted with the *N*-hydroxysuccinimide ester of palmitic acid in the presence of deoxycholate. The principal coupling product, palmitoyl-α-bungarotoxin, was purified with Sephadex LH-20 and phenyl-Sepharose column chromatography. The binding of palmitoyl-α-bungarotoxin with Triton-solubilized acetylcholine receptors showed specific binding with a dissociation constant of 2×10^{-7} M, which was approximately 20-fold lower in affinity than the native α-bungarotoxin. The fatty acid coupled toxin was incorporated into unilamellar liposomes (about 750 Å in diameter) by using a modified deoxycholate–gel filtration method. The toxin-containing liposomes, but not the protein-free liposomes, showed specific binding to the microsac membrane vesicles isolated from the electroplax tissue of *Torpedo californica* but not to the human red cell ghosts. The dissociation constant (2.6×10^{-7} M) for the liposome-bound toxin binding to the microsac vesicles was identical with that for the liposomal lipid binding to the same vesicles, indicating that the binding of both liposome components to the microsac vesicles was mediated by the same molecular event. The extent of liposome binding increased significantly when the toxin/lipid ratio of the liposomes was increased. Furthermore, the liposome-bound toxin molecules showed a dose-dependent inhibition of the agonist-dependent Na influx of the microsacs, indicating that the binding was mediated by specific interactions with the acetylcholine receptors. These results are discussed in terms of the multivalent binding between membranes.

Contact interactions between membranes play important roles in such diverse biological processes as cell recognition, adhesion, sorting, and fusion. These processes are often mediated by specific binding of membrane-bound receptors. Although the biological significance of these interactions has been recognized for a long time, the basic physicochemical studies only began recently. Pioneer theoretical works describing such interactions have been reported by Bell (1978, 1979) and others (De Lisi, 1980), and a number of explicit predictions remain largely unsubstantiated. We have decided to develop a simple experimental system which takes advantage of the much studied liposome model membrane. Our ultimate goal is to incorporate some well-defined receptor and ligand molecules into the surface membranes of liposomes and to study the liposome–liposome interactions as mediated by the binding of the receptor and ligand molecules. As a first step, we have chosen to incorporate a snake venom toxin, α-bungarotoxin (αBGT),[1] into liposomal membranes and to study their binding with the microsac membrane vesicles which are highly enriched with nicotonic acetylcholine receptors. The obvious advantage of using this ligand–receptor pair as a model system is its high specific binding affinity. In this communication, we report a method of coupling αBGT with fatty acid and the subsequent incorporation of the derivatized toxin to liposomes. We have also studied the binding of the toxin-bound liposomes with the microsac membrane vesicles and have demonstrated the potential usefulness for studies on membrane–membrane interactions.

Materials and Methods

Materials. αBGT was purified from the lyophilized venom of *Bungarus multicinctus* (Miami Serpentarium, FL) as described (Lee et al., 1972). The [^{125}I]iodo-BGT ([^{125}I]-αBGT) was prepared by using Chloramine T (Hunter & Greenwood, 1962). The specific activity of [^{125}I]-αBGT was 1.0×10^{13} cpm/mol. Hexadecyl [^{3}H]cholesteryl ether was synthesized and purified as described (Paltauf, 1968). The *N*-hydroxysuccinimide ester of palmitic acid (NHSP) was synthesized according to Lapidot et al. (1967) and recrystallized. [^{3}H]-NHSP was similarly prepared from [^{3}H]palmitic acid (New England Nuclear, Boston) with a final specific activity of 6.2×10^5 cpm/μg. NHSP was stored in anhydrous dioxane. Dioleoylphosphatidylcholine (DOPC) was purchased from Avanti (Birmingham, AL). All other chemicals were reagent grade.

Coupling of Palmitic Acid to αBGT. We have modified our previously published procedure (Huang et al., 1980) for the coupling of αBGT. Solvent-free NHSP (0.714 μmol) was mixed with [^{125}I]-αBGT (0.238 μmol) in 0.5 mL of phosphate-buffered saline (PBS), pH 7.4, containing 2% deoxycholate. The mixture was briefly sonicated in a bath sonifier and incubated at 37 °C for 3 h. Following the reaction, 1.5 mL of PBS was added to dilute the deoxycholate concentration to 0.5%.

Purification of Palmitoyl-αBGT (PBGT). The diluted reaction mixture was applied to a Sephadex LH-20 column (1.5 × 35 cm) preequilibrated and eluted with PBS. The fractions containing the ^{125}I-cpm peak were pooled and applied to a phenyl-Sepharose column (8-mL bed volume) preequilibrated with PBS. The column was first eluted with PBS, during which the unreacted αBGT was eluted. The eluting solvent was then changed to 50% EtOH in PBS to elute the fatty acid coupled αBGT. The radioactive protein fractions were pooled and stored at −20 °C. In an experiment in which [^{3}H]NHSP was used, the excess [^{3}H]palmitic acid from the

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[1] Abbreviations: αBGT, α-bungarotoxin; PBGT, palmitoyl-α-bungarotoxin; AchR, acetylcholine receptor; DOPC, dioleoylphosphatidylcholine; RBCG, red blood cell ghosts; PBS, phosphate-buffered saline; EDTA, disodium ethylenediaminetetraacetate.

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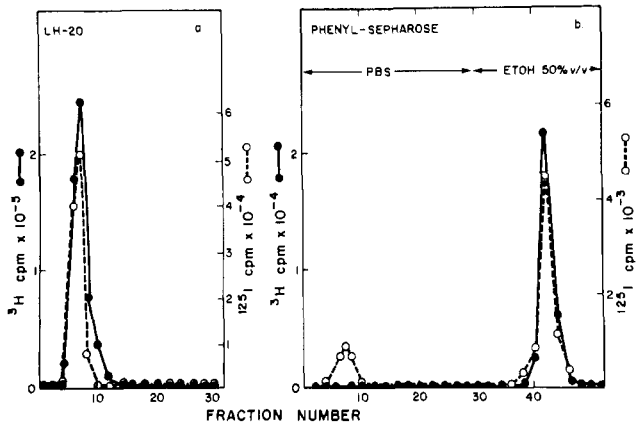

FIGURE 1: Purification of palmitoyl-αBGT. Crude reaction mixture of [^{125}I]-αBGT with [^{3}H]NHSP was eluted on a Sephadex LH-20 column (a). The pooled radioactive fractions from (a) were applied to a column of phenyl-Sepharose (b). ^{3}H cpm (●); ^{125}I cpm (○).

spontaneous hydrolysis of [^{3}H]NHSP was retained in the LH-20 column (Figure 1a). Also, the radioactive peak eluted by 50% EtOH in PBS in the phenyl-Sepharose column contained both ^{3}H and ^{125}I cpm (Figure 1b). The average molar ratio of palmitic acid to αBGT in this peak, calculated from the respective specific radioactivities, is approximately 1. We therefore concluded that the coupling product purified with this procedure was palmitoyl-αBGT (PBGT). The overall yield of PBGT after purification was about 60%. The purified PBGT only contained trace amounts of deoxycholate since most of the detergent was adsorbed in the LH-20 column matrix as measured by using [^{3}H]deoxycholate.

Preparation of Triton-Solubilized Acetylcholine Receptor (AchR). AchR was extracted from the frozen electroplax tissue of *Torpedo californica* (Pacific Biomarine, Venice, CA). The procedures of Karlin & Cowburn (1973) were used with modification. The homogenization buffer contained 0.2 M NaCl, and the pellet of the crude homogenate was washed 3 times with buffers containing 1 M NaCl. Extraction was done for 2 h at room temperature with 2% Triton X-100. All solutions contained 5 mM EDTA, 0.02% NaN_3, and 5 mM phenylmethanesulfonyl fluoride. The specific activity of the AchR preparation was about 1 nmol of toxin binding sites/mg of protein.

Binding of αBGT and PBGT to Detergent-Solubilized AchR. The detergent-solubilized AchR (5 μg of protein) in 50 μL of buffer containing 100 mM NaCl, 10 mM 3-(*N*-morpholino)propanesulfonic acid, 0.2% Triton X-100, and 100 μg/mL bovine serum albumin was added to various concentrations of [^{125}I]-αBGT or [^{125}I]PBGT in a final volume of 150 μL. Samples were incubated at 24 °C for 30 min. The amount of radioactive toxin–receptor complex was assayed by the procedure of Damle and Karlin (Damle & Karlin, 1978) with Whatman DE-81 filters.

Incorporation of PBGT into Liposomes. The method of Enoch & Strittmatter (1979) was used with modification. DOPC (10 mg/mL) with a trace amount of hexadecyl [^{3}H]cholesteryl ether (final specific activity 1.7×10^{12} cpm/mol of lipid) was sonicated for 30 min at 1 °C in a Heat Systems W-375 sonifier with a 0.5-in. probe. Various amounts of EtOH-free [^{125}I]PBGT in PBS containing 0.39% deoxycholate were added. The molar ratio of detergent to lipid was 0.75. The clear mixture was chromatographed on a Sephadex G-50 fine column (1 × 52 cm) preequilibrated and eluted with PBS. The fractions containing radioactive liposomes were pooled and dialyzed against PBS overnight in a Spectrapor-2 dialysis membrane (Spectrum Medical Ind., Los Angeles). The particulate matter in the dialyzate was removed by low-speed centrifugation. Approximately 90% of both lipid and protein were recovered.

Isolation and Radioiodination of Microsac Vesicles and Red Blood Cell Ghosts. The method of Miller et al. (1978) was followed to isolate the microsac membrane vesicles from frozen electroplax tissue of *T. californica*, except that the buffer used contained 400 mM NaCl, 5 mM EDTA, 0.02% NaN_3, 1 mM phenylmethanesulfonyl fluoride, and 10 mM sodium phosphate, pH 7.4. It had a specific activity of 0.1–0.5 nmol of toxin binding sites/mg of protein. Ghosts of human red blood cells were prepared as described (Hanahan & Ekholm, 1974). These membrane preparations were iodinated with ^{131}I (New England Nuclear, Boston) by the lactoperoxidase–glucose oxidase method (Hubbard & Cohn, 1972) to a final specific activity of 1×10^6 cpm/mg of protein. The toxin binding activity of the microsacs was not impaired by iodination.

Binding of Liposomes to Membrane Vesicles. ^{3}H-Labeled liposomes (0.4 mg of lipid) containing various amounts of [^{125}I]PBGT were incubated with ^{131}I-labeled microsacs or red blood cell ghosts (0.1 mg of protein) at 4 °C for 3 h in PBS. The mixture was then spun in an SW 50.1 rotor at 190000*g* for 135 min at 4 °C through a 10% (w/v) sucrose solution. This procedure pelleted >90% of the microsacs or red blood cell ghosts. The pellets were washed twice in PBS before counting for ^{3}H, ^{125}I, and ^{131}I. The extent of liposome binding to membrane vesicles (^{3}H and/or ^{125}I cpm) in the pellet was corrected for microsacs and ghost recovery as measured by ^{131}I cpm.

^{22}Na Influx Measurements of Microsac Vesicles. Microsac vesicles (35 μg of protein/μL) were mixed with an equal volume of buffer (400 mM NaCl, 1 mM EDTA, 10 mM NaN_3, and 10 mM sodium phosphate, pH 7.5) containing various concentrations of αBGT or PBGT–liposomes. After incubation for 2 h at 4 °C, 10–20-μL aliquots were assayed in triplicate for carbamoylcholine stimulation of ^{22}Na influx into the microsac vesicles as described (West & Huang, 1980). The amount of ^{22}Na in the microsacs after a 10-s influx period was determined by a Millipore filtration method (West & Huang, 1980). The concentration of carbamoylcholine (Sigma) used was 0.2 mM.

Other Analytical Methods. Lipid phosphorus was determined by the method of Ames & Dubin (1970) for the phospholipid quantitation. Protein was determined by the method of Lowry et al. (1951). ^{125}I and ^{131}I were counted in a Beckman Biogamma II counter. ^{3}H was counted in a Beckman LS-230 liquid scintillation counter by using a Triton X-100 containing cocktail. All results were corrected for channel crossover if more than one isotope was used.

Results

Acylation of αBGT. We have used an activated ester of a fatty acid for the coupling of αBGT with palmitic acid. The principal coupling product, palmitoyl-αBGT, was purified by hydrophobic column chromatography with satisfactory yield (Figure 1). Although the precise position of coupling on the protein is not known, it is likely that the ε-NH_2 group of Lys-51 of the toxin is acylated, since the acylation of the same group by a similar reagent, i.e., *N*-succinimidyl propionate, has been suggested (Dolly et al., 1981). We found that a ratio of 3:1 of NHSP to αBGT was optimal for the generation of monoderivatized product; higher ratios yielded multiderivatized product, and lower ratios did not give a good yield of PBGT. The half-life of NHSP hydrolysis at 37 °C was about 2 h (B. P. Babbitt, unpublished observation).

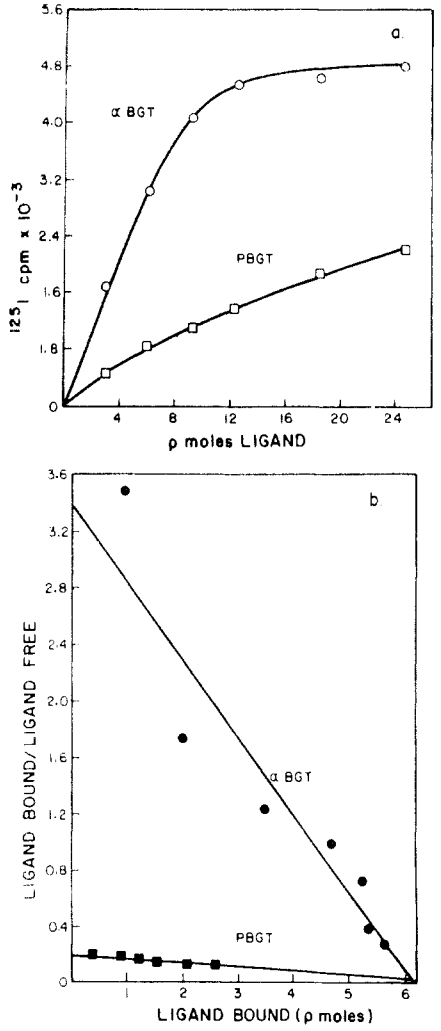

FIGURE 2: Binding of αBGT (○ and ●) and PBGT (□ and ■) to Triton X-100 solubilized AchR (a) and Scatchard plots of data (b). Straight lines in (b) are least-squares fits of the data with correlation coefficients of 0.97 for αBGT and 0.99 for PBGT.

Although the native αBGT is a very hydrophilic protein, addition of one palmitoyl chain per protein molecule has rendered the molecule much more hydrophobic. This was evident from the observation that PBGT was much more soluble in EtOH than the native αBGT. PBGT is insoluble in aqueous buffer. At least a small amount of deoxycholate (>0.01%) is necessary to clarify the PBGT solutions.

Binding Affinity of PBGT with Detergent-Solubilized AchR. A direct measurement of the binding of PBGT with the Triton-solubilized AchR was done. Figure 2a shows the results of binding of PBGT as well as those of αBGT. When the same data are analyzed in a Scatchard plot (Figure 2b), the apparent dissociation constants, K_D, are 2.06×10^{-7} M and 1.16×10^{-8} M for the PBGT and αBGT, respectively. Thus, the addition of one palmitoyl chain to αBGT decreased the binding affinity of the molecule by about 20-fold. It is further evident from Figure 2b that the PBGT and αBGT are bound to the same class of receptor, since both lines extrapolate to the same point in the horizontal axis. The specific activity of AchR used in this experiment can be calculated to be 1.2 nmol of toxin binding sites/mg of protein. Furthermore, the Scatchard plot for PBGT indicates one single type of binding. We take it as further evidence for the homogeneity of the PBGT preparation.

Incorporation of PBGT into Liposomes. The method using deoxycholate and gel filtration was successfully modified for the incorporation of PBGT into liposomes. The method is

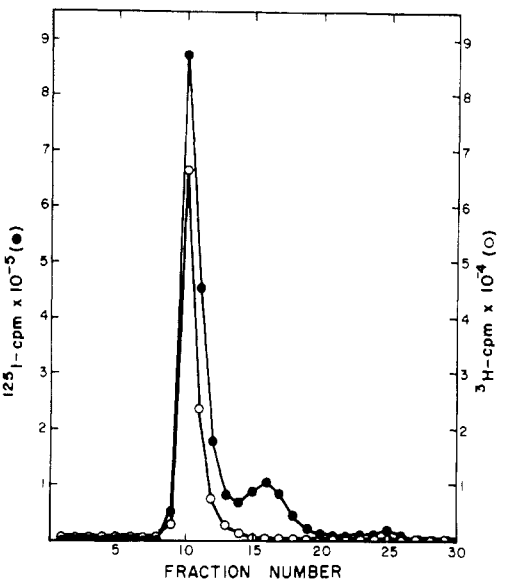

FIGURE 3: Incorporation of [^{125}I]PBGT (●) into liposomes labeled with hexadecyl [^{3}H]cholesteryl ether (○). Liposomes were eluted at the void volume and separated from the unincorporated PBGT (second peak). PBGT (18.63 nmol) and dioleoylphosphatidylcholine (3.726 μmol) were used.

especially suitable for this purpose since PBGT requires a low concentration of detergent to remain in solution. Although the bulk of deoxycholate was removed by gel filtration, the remaining deoxycholate must be removed by dialysis. Figure 3 shows an elution profile of a Sephadex G-50 fine column. The void fractions contained liposomes with incorporated PBGT, which was separated from the excess deoxycholate and the unincorporated PBGT. We routinely recovered about 90% of both PBGT and lipids with this procedure. The resulting liposomes were primarily unilamellar with an average diameter of 750 Å as determined by negative-stain electron microscopy (photograph not shown). The amount of PBGT incorporated into liposomes could be varied by changing the ratio of lipid to PBGT in the detergent-solubilized mixture. Regardless of the amount incorporated, the resulting liposomes had approximately the same size distributions and average diameters. αBGT did not incorporate into liposomes when tested with an identical procedure (chromatogram not shown). Additional evidence for the association of PBGT with liposomes after the column procedure came from the result of sucrose gradient centrifugation. When PBGT–liposomes were centrifuged at 190000*g* for 135 min in a linear 5–20% (w/v) sucrose gradient, both PBGT and lipids cosedimented at the top of the gradient. When PBGT alone was tested in an identical condition, >95% of PBGT pelleted.

Binding of PBGT–Liposomes with Microsacs. A series of liposome preparations containing various amounts of [^{125}I]-PBGT and a trace of hexadecyl [^{3}H]cholesteryl ether as a marker for liposomal lipids was tested for binding with microsac membrane vesicles. In this experiment, a constant amount of liposomal lipids with various amounts of PBGT was added to a fixed amount of microsacs. RBCG were used as a control for nonspecific binding. After equilibration, the bound liposome was separated from the unbound by centrifugation on a sucrose step gradient. Figure 4 shows the amount of binding for both [^{125}I]PBGT and ^{3}H-labeled lipids as a function of the PBGT density in the applied liposomes. At low densities of PBGT in liposomes, little binding of both toxin and lipids was detectable. However, a significant amount of binding occurred at higher densities of PBGT in liposomes. Liposome binding to microsacs increased continuously with

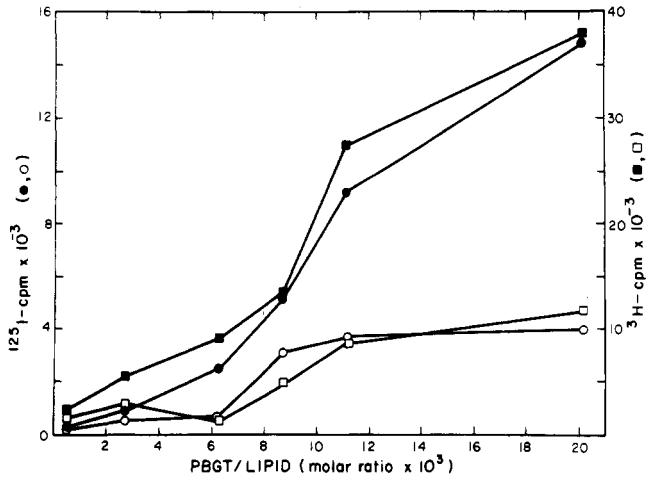

FIGURE 4: Binding of PBGT–liposomes with microsac vesicles or human red blood cell ghosts. Liposomes (2.76×10^4 pmol and 1.9×10^5 cpm of total ^{3}H-labeled lipids) containing various amounts of [^{125}I]PBGT were added to 100 μg of microsacs (● and ■) or ghosts (○ and □). The binding of both ^{125}I cpm (● and ○) and ^{3}H cpm (■ and □) was measured.

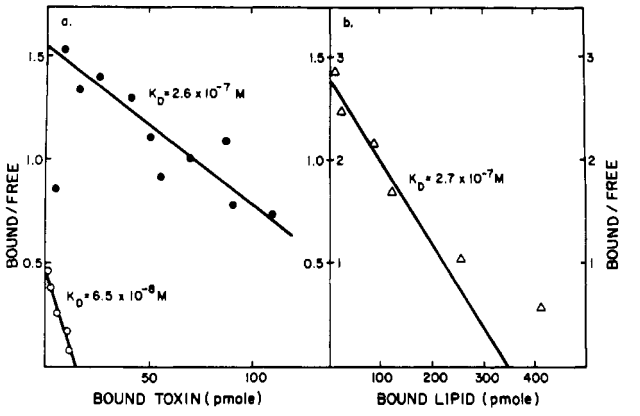

FIGURE 5: Scatchard analyses of binding of PBGT–liposomes (protein/lipid = 0.005) and native αBGT to microsac vesicles (100 μg of protein). The binding of αBGT (○) and liposome-bound PBGT (●) is shown in panel a, and binding of liposomal lipids (Δ) of the PBGT–liposomes is shown in panel b. Straight lines are least-squares fits of data points with correlation coefficients greater than 0.9.

the increasing densities of PBGT in liposomes. The binding of toxin paralleled that of the liposomal lipids, indicating that the intact liposomal structures were bound to microsacs. Nonspecific binding of liposomes to RBCG was only slightly enhanced at high toxin densities. The equilibrium binding of a toxin-containing liposome preparation (protein/lipid = 0.005) to the microsac vesicles was also measured at different liposome concentrations. The result of the saturation binding shown in Figure 5 indicates that the dissociation constant of this liposome-bound toxin binding ($K_D = 2.6 \times 10^{-7}$ M) was identical with that of the liposomal lipid binding ($K_D = 2.7 \times 10^{-7}$ M). This result strongly suggests that the binding of toxins and lipids to the microsac vesicles was mediated by the same molecular event, most likely the binding of toxin to the acetylcholine receptors on the microsac vesicles. In a parallel experiment, the binding of αBGT to the same microsac vesicles showed a K_D of 6.5×10^{-8} M (Figure 5a).

Inhibition of Agonist-Induced ^{22}Na Influx of Microsacs by PBGT–Liposomes. In order to demonstrate that the binding of PBGT–liposomes to microsacs was of a specific nature, i.e., the binding was mediated by the acetylcholine receptors in the microsacs, we have measured the inhibition of the carbamoylcholine-induced ^{22}Na influx into microsac vesicles by the PBGT–liposomes. Two different preparations of PBGT–liposomes with protein/lipid molar ratios of 0.1 and 0.063 were prepared. Free, underivatized αBGT was used for comparison. The carbamoylcholine-induced ^{22}Na influx of microsacs was blocked in a dose-dependent manner by pretreatment with αBGT, with a complete inhibition at about 0.1 nmol (Figure 6A). PBGT–liposomes with a lower molar ratio (0.063) also inhibited the influx, but only partial inhibition (about 60%) was observed even at the highest toxin dose (0.22 nmol) used (Figure 6B). For liposomes with a higher ratio (0.1), complete inhibition was detected at about 0.1 nmol (Figure 6C). These results are in agreement with the observation that the binding affinity of liposomes is a direct function of the toxin density in liposomes. The fact that PBGT–liposomes can inhibit the agonist-induced ^{22}Na influx provides direct support to the conclusion that liposome binding is mediated by the specific interaction of the toxin with the acetylcholine receptors.

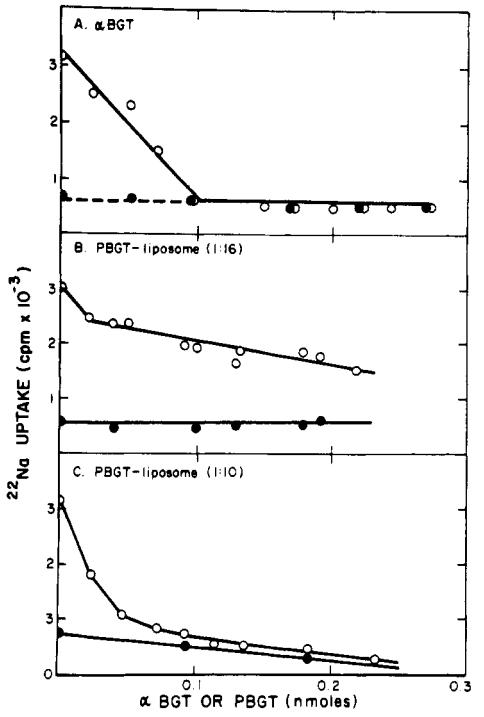

FIGURE 6: Inhibition of ^{22}Na influx of microsacs by pretreatment with αBGT (A) or PBGT–liposomes (B and C). ^{22}Na uptake in the absence (●) or presence (○) of carbamoylcholine was measured after pretreatment with the indicated amount of toxin in 10 μL (A and B) or 20 μL (C). The PBGT/lipid molar ratio was 1:16 in (B) and 1:10 in (C).

Discussion

We have chosen the acetylcholine receptor–αBGT system to develop a model for the study of specific membrane interactions. This system has several advantages: (1) both the receptor and the toxin can be readily purified, (2) the biochemical and biophysical characteristics of the system have been well studied, (3) the binding between the toxin and the receptor is highly specific, and (4) the physiological activity of the system is well-defined (Heidmann & Changeaux, 1978). These distinct advantages are likely to provide detailed physicochemical information about the specific interactions between membranes. However, this system does require the attachment of the αBGT to some membrane system before it can be used. The well-defined liposome model membranes are chosen for this purpose. αBGT is a highly water soluble protein with no significant ability to bind with liposomes. We therefore decided to covalently attach a hydrophobic anchor to the toxin molecule and expected the incorporation of the amphipathic toxins into liposomal membranes.

Previously, we have shown that IgG antibody can be covalently coupled with palmitic acid and the palmitoyl-IgG can be readily incorporated into liposomes by using the detergent deoxycholate (Huang et al., 1980). In the present study, NHSP is successfully used to couple palmitic acid to αBGT. The principal product, PBGT, was purified by hydrophobic chromatography with satisfactory yield. The purified PBGT readily incorporated into unilamellar liposomes of medium size (about 750 Å) with the use of deoxycholate and a gel filtration method. More importantly, the purified PBGT still binds specifically with the detergent-solubilized acetylcholine receptor, although with somewhat lower affinity (Figure 2). We therefore expect that the PBGT–liposomes and the acetylcholine receptor containing microsac vesicles would serve as a good model for studies of specific membrane interactions.

One of the important features for such interactions, e.g., cell recognition, is the multivalent nature of the binding system. We have examined this property by systematically varying the protein/lipid ratio of the liposomes. Indeed, we have observed a continuous increase in the apparent binding of liposomes to microsacs as the protein/lipid ratio of the liposome increases (Figure 4). In the range of ratios tested in this experiment, the apparent affinity of liposomes has increased about 20-fold. Compared to liposomes without toxin which do not bind with microsacs, this level of binding is quite impressive. Furthermore, when the liposome-bound PBGT was tested for the ability to inhibit the agonist-dependent ^{22}Na influx of the microsac vesicles, the toxin potency depended on the toxin/lipid ratio of the liposomes (Figure 6). At a low ratio (0.063), only partial inhibition was observed. However, liposomes with a higher ratio (0.1) could completely block the ion channel activity of microsacs with an efficiency comparable to that of the native αBGT. This result is in direct contrast with the binding data shown in Figure 2 in which the binding affinity of PBGT to the detergent-solubilized AchR is shown to be about 20-fold lower than that of the αBGT. Although the binding affinity of the PBGT–liposomes used in the experiment shown in Figure 6 was not determined, our data suggested that the apparent affinity of the lipsome-bound PBGT could be markedly increased to a level comparable to that of the αBGT by simply attaching a sufficient amount of toxin molecules on the liposome surface. This observation is in agreement with that seen with antibody–antigen binding. The apparent affinity of a bivalent antibody molecule (e.g., IgG) is about 1–2 orders of magnitude higher than that of the corresponding monovalent molecule (e.g., Fab) (Karush, 1976). Therefore, a relatively weak binder can be turned into a significantly stronger one by making it multivalent.

The observed binding of PBGT–liposomes to microsacs is specific with respect to the acetylcholine receptor molecules. First of all, the binding of PBGT–liposomes to the human red blood cell ghosts was not significant even at high PBGT/lipid ratios. Much of the observed nonspecific binding is probably due to the interactions between the postively charged toxins and the negatively charged cell surfaces. Furthermore, the binding of liposomal lipids to the microsac vesicles showed an identical K_D with that of the liposome-bound toxins (Figure 5), indicating that the same molecular event was responsible for the binding of both liposome components. The most convincing evidence for specific binding stems from the experiments shown in Figure 6 in which the agonist-dependent ^{22}Na influx of the microsacs was inhibited by the PBGT–liposomes. The fact that PBGT–liposomes not only bind with microsacs but also block the ionophore activity of the acetylcholine receptors directly indicates that the binding of liposomes with microsacs is mediated by the specific interactions of PBGT with the receptors.

In the present studies, the percentage of toxin molecules in PBGT–liposomes actually exposed on the liposome surface is not known. If it is assumed that PBGT is randomly distributed on the two sides of the bilayer and there are about 5×10^4 phospholipid molecules per 750-Å liposome (Enoch & Strittmatter, 1979), a toxin/lipid ratio of 0.01 (the routine ratio we used) would correspond to about 2.5×10^2 surface-exposed PBGT molecules per liposome. The surface density of PBGT would be about 1.4×10^4 molecules/μm^2. The surface density of AchR in the microsac membrane vesicles is about $10^4/\mu m^2$ (Heuser & Salpeter, 1979). Whether the density of toxin in liposomes must match or exceed that of the receptor in microsacs to achieve significant binding of liposomes is an interesting question and will be a subject of further experimentation.

Bell (1978) in his theoretical work on membrane recognition has predicted that the binding of membranes is a sensitive function of the receptor mobility in the membranes under specified conditions. In the present model system, PBGT is most likely anchored to the lipid bilayer by a single fatty acyl chain. One would expect a considerable amount of rotational freedom of the toxin molecules on the liposome surface as compared to that of an integral membrane protein which is more or less embedded in the lipid bilayer. Furthermore, the liposomes used in this study were made of dioleoylphosphatidylcholine, an unsaturated lipid which gives fluid bilayers at the experimental temperature (4 °C). Therefore, the lateral mobility of the toxin molecules on the liposome membrane would be fairly high. In contrast, the AchR's in the microsac membranes are transbilayer proteins (Huang, 1979; Ross et al., 1977; Klymkowsky & Stroud, 1979). The lateral mobility of AchR at the myotube cell surface is quite restricted with a diffusion coefficient of only $(4–5) \times 10^{-11}$ cm^2/s (Axelrod et al., 1976). The rotational diffusion of AchR in microsac vesicles of *Torpedo marmorata* has been measured by the EPR technique and was found to be essentially immobile (rotational correlation time $> 10^{-3}$ s) (Rousselet & Devaux, 1977). Whether a relatively mobile ligand (PBGT) can bind efficiently with a relatively immobile receptor (AchR) is another interesting question to which the present model system could offer a definitive answer. We are currently preparing PBGT–liposomes with more "rigid" lipids such as dipalmitoylphosphatidylcholine in an attempt to decrease the lateral mobility of the toxin molecule. These studies offer an opportunity to explore some physicochemical characteristics of specific membrane interactions of some fundamental importance.

In conclusion, we feel that we have developed a simple model system for studies on specific membrane interactions. The system is well-defined both biochemically and biophysically and offers a great flexibility and diversity necessary for detailed mechanistic studies.

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Structure of the *Escherichia coli* K2 Capsular Antigen. Stereochemical Configuration of the Glycerophosphate and Distribution of Galactopyranosyl and Galactofuranosyl Residues†

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ABSTRACT: The *Escherichia coli* K2 capsular antigen is known to be composed of α-D-galactopyranosyl(1–2)glycerophosphate and α-D-galactofuranosyl(1–2)glycerophosphate units which are connected by phosphodiester bonds to C-4 of the galactopyranosyl and C-5 or C-6 of the galactofuranosyl moieties. In the present study the glycerophosphates were released by two different procedures and shown to have the *sn*-glycero-3-phosphate stereochemical configuration. In the first, the chain was fragmented by Smith degradation to glycerophosphothreitol from which the glycerophosphate was released by alkali hydrolysis. The structure-dependent low recovery of α-glycerophosphate (<10%) initiated the development of another degradative sequence which consisted of periodate oxidation, β elimination, hydrazinolysis, and alkaline treatment. This way, approximately 90% of the glycerophosphate was released as *sn*-glycero-3-phosphate. β elimination revealed in addition that most of the galactofuranosyl residues carry the phosphodiester bond at position 5. Separation by gel permeation chromatography and analysis of the fragments obtained by β elimination showed that pyranosidic and furanosidic galactosyl residues alternate in the same chain and suggested the sequences Gal*f*(*p*)GroP–(Gal*p*GroP)$_n$–Gal*f*– and –Gal*f*GroP–(Gal*p*GroP)$_n$–Gal*f*–, where *n* is 6, 4, and 3, respectively.

The occurrence of teichoic and lipoteichoic acids appeared to be confined to Gram-positive bacteria [for reviews, see Knox & Wicken (1973) and Lambert et al. (1977)] until recent investigations into the capsular antigen of *Escherichia coli* K2 and K62 revealed a poly(galactosyl(α1–2)glycero-1(3)-phosphate) structure (Jann et al., 1980; Jann & Schmidt, 1980) which was closely related to the poly(glycosylglycerophosphate)-containing types of teichoic (Burger & Glaser, 1966; Archibald & Coapes, 1971) and lipoteichoic acids (LTA)[1] (Koch & Fischer, 1978). In the previous work on these capsular antigens, the stereochemical configuration of the glycerophosphate residues was not investigated, and the arrangement of the pyranosidic and furanosidic galactosyl residues which were observed in both polymers remained open. Here we report on these two structural features of K2 antigen.

Materials and Methods

Materials. Escherichia coli K2 capsular antigen was prepared as previously described (Jann et al., 1980). Lipoteichoic acid from *Streptococcus lactis* Kiel 42172 stemmed from earlier work (Koch & Fischer, 1978). Cyclic 1(3),2-glycerophosphate was prepared according to the procedure of Ukita et al. (1955), erythritol phosphate by reduction of D-erythrose 4-phosphate with $NaBH_4$, and di-α,α′-glycerol phosphate by deacylation of phosphatidylglycerol (Fischer et al., 1973).

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[1] Abbreviations: Gal*p*, α-D-galactopyranosyl; Gal*f*, α-D-galactofuranosyl; GroP, *sn*-glycero-3-phosphate residue; LTA, lipoteichoic acid.

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