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Binding of Immunoglobulin G to Phospholipid Vesicles by Sonication†

Leaf Huang* and Stephen J. Kennel

ABSTRACT: Purified goat immunoglobulin G (IgG) does not bind to sonicated phospholipid vesicles. However, when IgG is sonicated together with phospholipids, 4–40% of the IgG can be bound to the vesicles, depending on the experimental conditions. The extent of binding depends on the period and power of sonication, the IgG to lipid ratio, and the lipid composition. Anionic phospholipids such as phosphatidylglycerol and phosphatidylserine, but not cholesterol, enhance binding about 50% over that obtained with the neutral phosphatidylcholine. Binding of IgG causes extensive aggregation of vesicles, as shown by electron microscopy, and aggregates can be separated from unbound IgG by molecular-sieve chromatography on Sepharose 4B or by sucrose density gradient centrifugation. The IgG–vesicle aggregates remain stable in either phosphate-buffered saline or 50% fetal calf serum up to 20 h at 37 °C, although substantial lipid degradation in 50% fetal calf serum was observed. The use of goat IgG containing antibody to a purified protein antigen allowed quantitation of antibody activity of these preparations. Immune IgG sonicated alone shows 100% of the original antigen binding capacity while vesicle-bound IgG retains 30–50%. Antigen binding capacity of bound IgG is not increased when vesicles are lysed by 1.5% NP-40, suggesting all of the bound IgG is exposed on the outer surfaces of the vesicles. IgG's of human, mouse, and rabbit, as well as the purified goat $F(ab')_2$ fragments, also bind with vesicles by cosonication.

Phospholipid vesicles (liposomes) have been used extensively both to modify the plasma membrane composition and cell behavior and as carriers for drugs, hormones, enzymes, or other biologically active substances in vitro and in vivo (see reviews, Pagano & Weinstein, 1978; Gregoriadis, 1976a,b). One of the central problems of this area of research is the lack of specificity of the interaction between phospholipid vesicles and cells. It is particularly serious for in vivo experiments in which intravenously injected vesicles are rapidly cleared from circulation by the reticuloendothelial cells in liver and spleen (Juliano & Stamp, 1975; Gregoriadis & Neerunjun, 1974), thus preventing the effective use of vesicles as pharmaceutical carriers for other target tissues. Attempts have been made to modify the vesicle surface membranes with some "recognition" molecules so that vesicles can acquire specificity in terms of the type of tissue with which they interact (Gregoriadis & Neerunjun, 1975; Weinstein et al., 1978). Among all "recognition" molecules, immunoglobulins are preferred because of their ease of preparation, relatively high binding constants, specificity of recognition of target molecules, and ability to bind different antigen molecules. It has been shown that phospholipid vesicles coated with IgG[1] antibody can deliver their entrapped contents to target cells significantly better than uncoated vesicles (Gregoriadis & Neerunjun, 1975). Vesicles coated with heat aggregated IgM also cause

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[1] Abbreviations used: IgG, immunoglobulin G; PC, L-α-phosphatidylcholine; PG, α-phosphatidyl-DL-glycerol; chol, cholesterol; TLC, thin-layer chromatography; gp70, major envelope glycoprotein of Maloney leukemia virus; PBS, phosphate-buffered saline; αBGT, α-bungarotoxin; NP-40, Nonidet P40.

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enhanced phagocytic uptake of the entrapped horseradish peroxidase to dogfish phagocytes (Weissmann et al., 1975). These initial successes in using immunoglobulins to target phospholipid vesicles to specific cell types have prompted us to study in some detail the binding of immunoglobulins to the vesicle membranes. We report here that IgG can be bound to vesicles by sonication and that the vesicle-bound IgG retains a substantial amount of its antigen binding capacity.

Materials and Methods

Lipids. Phosphatidylcholine (PC) was purified from hen egg yolk following published procedures (Huang & Pagano, 1975). [^{3}H]Dipalmitoyl-PC was prepared by a catalytic tritation of dipalmitoleyl-PC and subsequently purified by silicic acid chromatography (Huang & Pagano, 1975).

Cholesterol and L-α-phosphatidyl-DL-glycerol (PG) were purchased from Sigma. Phosphatidylserine was obtained from Supelco. All lipids were stored in chloroform at −70 °C under N_2. The purity was checked by thin-layer chromatography (TLC). Phospholipid was assayed by lipid phosphorus determination (Ames & Dubin, 1960).

IgG and gp70. The major envelope glycoprotein of Moloney leukemia virus, gp70, was purified from whole virus by solubilization with KBr and subsequent phosphocellulose chromatography (Kennel, 1976). IgG from a goat immunized with purified gp70 (immune IgG) and normal IgG were prepared as previously described (Kennel, 1976). F(ab′)$_2$ fragments were prepared by digesting immune IgG (20 mg/mL) in 0.1 M sodium acetate buffer, pH 4.0, with 0.5 mg/mL of pepsin (Worthington) for 18 h at 37 °C. The fragments were purified by gel filtration following neutralization of the digest and found to contain the same antigen binding capacity per mole as the starting IgG. Gel electrophoresis showed less than 1% contamination with undigested IgG. IgG's of mouse, rabbit, and human were purified from normal sera using procedures similar to those used for goat IgG. The gp70 was radioiodinated with ^{125}I to a specific activity of about 40 μCi/μg using chloramine-T and stored at 4 °C at a concentration of 100 ng/mL in 5 mg/mL of bovine serum albumin and 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4 (PBS). IgG was radiolabeled with ^{131}I to a specific activity of 10–20 μCi/mg using chloramine-T. This level of iodination does not impair the antigen binding capacity of IgG (Sonada & Schlamowitz, 1970). Protein concentration was measured by the Lowry method (Lowry et al., 1951).

Preparation of Phospholipid Vesicles. Known amounts of lipid plus trace [^{3}H]dipalmitoyl-PC (final specific activity about 0.1 μCi/μmol) were mixed in chloroform. Organic solvent was removed under a stream of N_2 and then with vacuum so that a thin film of dry lipid was deposited at the bottom of a test tube. Phosphate-buffered saline (PBS), with or without IgG (0.1–1 mg/mL), was added and the mixture was vortexed or briefly sonicated in a bath-type sonifier to suspend the lipids. The milky suspension was then transferred to a water-jacketed glass vessel and sonicated in a probe-type sonifier (Bronson S-110 with half-inch horn) under argon at 1 °C for various power settings and time intervals as specified in the text. After sonication, the slightly turbid solution was centrifuged at 11000g for 10 min to remove metal fragments released from the tip of the sonifier probe. The supernatant was immediately analyzed for IgG binding or used for other experiments.

Molecular-Sieve Chromatography. Two sizes of Sepharose 4B columns were used for preparative and analytical fractionation of vesicle samples. Preparative samples of sonicated phospholipid vesicles (about 5 mL total), with or without bound IgG, were fractionated on 2.6 × 29 cm columns by elution with PBS (~1.3 mL/min, 2-mL fractions). Analytical samples (0.1–0.5 mL) were fractionated on 0.8 × 20 cm columns in Falcon plastic pipets by elution with PBS (with or without 1% Triton X-100, 0.2 mL/min, 0.15–0.5-mL fractions). Blue Dextran (Sigma) was used as a void volume marker for both types of column chromatography. In some cases, α-[^{125}I]-bungarotoxin (prepared as cited in Devreotes & Fambrough, 1975) was also used as a marker. The recoveries of IgG and lipids in these columns were about 90 and 80%, respectively.

Sucrose Density Gradient Centrifugation. Aliquots (200 μL) of the IgG–vesicle complexes eluted at the void volume of the Sepharose 4B column were immediately overlaid on a 4.5-mL linear sucrose gradient (5–20% w/w in PBS). As a control, an equal volume mixture of presonicated IgG and presonicated vesicles, after incubation at 37 °C for 30 min, was also overlaid on an identical gradient. Tubes were centrifuged at 48 000 rpm in an SW 50.1 rotor for 7 h at 4 °C. Fractions of 10 drops were collected from the bottom of the tube and counted in a liquid scintillation counter for both ^{3}H and ^{131}I.

Antigen Binding Assay. Experiments were done by incubating 1 ng of [^{125}I]gp70 plus dilutions of ^{131}I-labeled immune IgG with carrier IgG (10 μg of IgG total) for 4 h at 37 °C in a volume of 600 μL containing 5 mg/mL of bovine serum albumin and, in some cases, 1.5% Nonidet P40 (NP-40). Immune complexes were precipitated by incubation with excess rabbit antiserum to goat IgG for 1 h. The precipitates were collected by centrifugation, washed with PBS, and counted for both ^{125}I and ^{131}I in a two-channel γ counter.

Electron Microscopy. Vesicle-bound IgG was negatively stained with 1% potassium phosphotungstate, pH 7.2, and viewed in a JEM 6c electron microscope, operating at 80 kV. Pictures were taken at 40 000 magnification and further enlarged photographically.

Thin-Layer Chromatography. Pooled fractions from Sepharose 4B column eluants were extracted with equal volumes of $CHCl_3$/MeOH (2:1, v/v) and the organic phase condensates spotted on silica gel G plates. The developing solvent was $CHCl_3$/MeOH/H_2O (65:35:5, v/v). Lipid spots on the plates were visualized by I_2 vapor. Zones of 0.5 cm were scraped from the plates and counted for ^{3}H radioactivity. Identification of lipid species was aided by authentic standards.

Results

IgG Binding to Vesicles Requires Sonication. When IgG and a mixture of PC, cholesterol, and PG (mole ratio 8.5:2.4:1.3) were sonicated together and subjected to Sepharose 4B column chromatography, about 20% of the IgG, together with some ^{3}H-labeled phospholipids, was eluted at the void volume of the column (Figure 1c). IgG preparations sonicated alone did not contain material eluting at the void volume (Figure 1a). Furthermore, no binding was observed when presonicated IgG and presonicated vesicles were incubated together at 37 °C for 30 min (Figure 1d). Unsonicated IgG also did not bind with vesicles (not shown). Therefore, the binding was dependent on the presence of both components during sonication.

To confirm the binding between IgG and vesicles, the void volume eluant of the Sepharose 4B column was analyzed by sucrose density gradient centrifugation (data not shown). All of the [^{131}I]IgG sedimented as a broad peak of 8–13 S. There was about two-thirds of the total ^{3}H-labeled lipids cosedimented with this peak. The rest of ^{3}H-labeled lipids sedimented at the top of the gradient, probably representing excess unbound vesicles. The control gradient on a mixture of

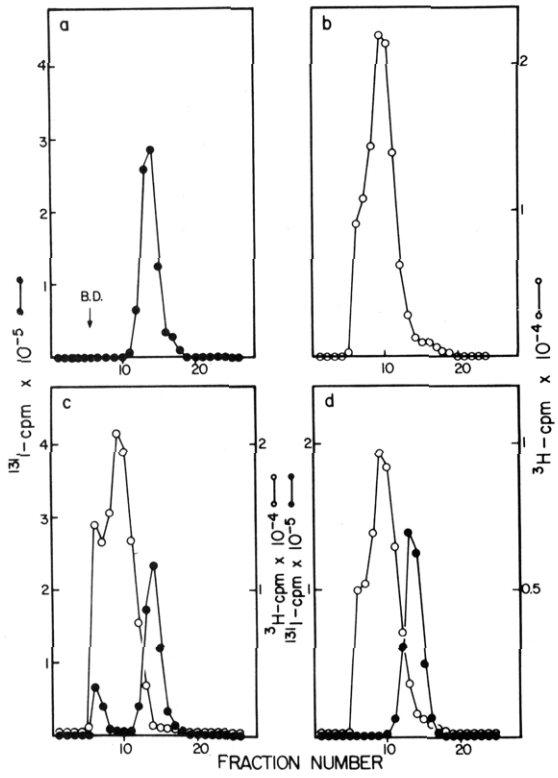

FIGURE 1: Elution profiles of Sepharose 4B chromatography. Samples were (a) [^{131}I]IgG (0.5 mg/mL) sonicated alone; (b) ^{3}H-labeled lipid (PC/chol/PG, 8.5:2.4:1.3 μmol/mL) sonicated alone; (c) IgG and lipids sonicated together; and (d) equal volume mixture of a and b incubated 30 min at 37 °C. Sonication was 25 min at power setting 1. ^{131}I-cpm (●) and ^{3}H-cpm (○).

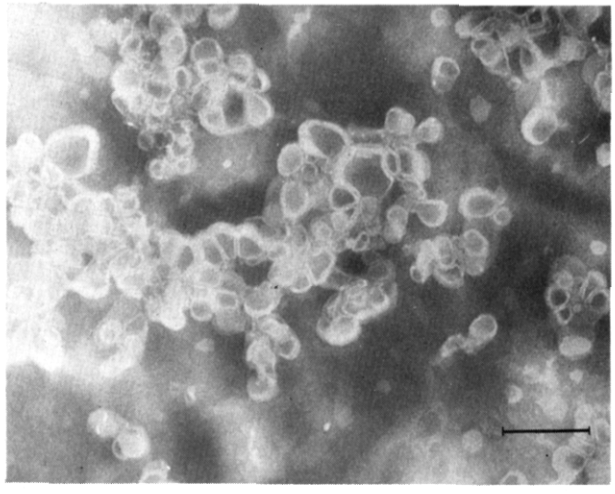

FIGURE 2: Electron micrograph of negatively stained IgG–vesicle complex. Bar is 2000 Å.

presonicated IgG and presonicated vesicles showed complete separation of the two components, indicating no binding in this condition.

Since the majority of IgG–vesicle complexes were eluted at the void volume of Sepharose 4B columns, some aggregation of the IgG-bound vesicles was suspected. This was confirmed when the complexes were examined by electron microscopy. Negatively stained samples from the void volume eluant showed extensive clumping (few to few hundred vesicles per clump) of vesicles ranging in diameter from 250 to 1500 Å (Figure 2). If the void column material from the same lipid mixture sonicated without IgG was similarly examined, only non- or slightly aggregated vesicles of 600–1200 Å in diameter were seen (picture not shown).

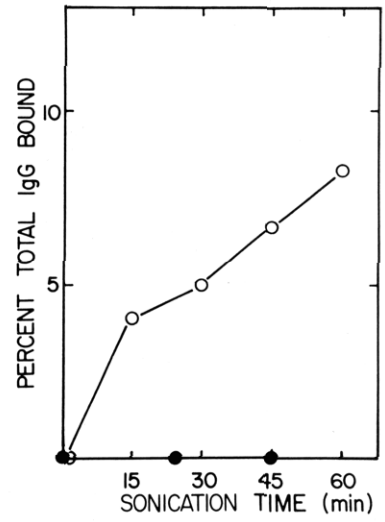

FIGURE 3: Effect of sonication time on IgG–vesicle binding. [^{131}I]IgG (0.1 mg/mL) was sonicated with a lipid mixture (PC/chol/PG, 0.85:0.24:0.13 μmol/mL) at power setting 1. The percent of total IgG eluted at the void volume of a Sepharose 4B column is plotted against sonication time. IgG sonicated with lipids (○) or sonicated alone (●).

Table I: Dependence of Lipid Composition on IgG–Vesicle Binding

lipid (μmol/mL)	% total IgG bound[a]
PC (12.3)	21
PC/chol (9.9:2.4)	23
PC/PG (10.9:1.3)	32
PC/chol/PG (8.5:2.4:1.3)	35

[a] [^{131}I] IgG (0.1 mg/mL) was sonicated with lipids for 30 min at power setting 1. The sonicate was chromatographed on Sepharose 4B to separate the bound IgG from unbound IgG.

Optimal Conditions for Binding. The degree of IgG binding to vesicles was dependent on sonication conditions. First, we noticed that a higher degree of binding was obtained if the power setting of the sonifier was higher. For example, in one experiment, 17% of the total IgG was bound to vesicles when sonicated 25 min at power setting 1, whereas 31% binding was obtained when an identical sample was sonicated at power setting 2. Binding was also dependent on sonication time. Figure 3 shows the result of an experiment in which 0.1 mg/mL of IgG was sonicated with 1.23 μmol/mL of a PC/chol/PG mixture at power setting 1 for various time intervals. The binding of IgG to vesicles increased with increasing sonication time. No IgG was eluted at the void volume after sonication by itself for periods up to 45 min.

The binding of IgG to vesicles was also dependent on the composition of the lipid. Table I shows the results of a typical experiment in which IgG was sonicated with lipids of various composition while the total lipid concentration was kept constant (12.3 μmol/mL). PC alone formed vesicles which bound about 20% of the total IgG. Addition of cholesterol (20 mol %) did not alter this binding. However, when an anionic lipid, PG, was added to either PC or PC/chol, about 50% more binding was obtained. Another anionic phospholipid, phosphatidylserine, produced similar enhanced binding of IgG to vesicles (not shown). The ratio of IgG to total lipid in the sonication mixture was also important to the final degree of IgG binding. When 0.1 mg/mL of IgG was sonicated for 30 min with increasing concentration of lipid mixture (PC/chol/PG), the amount of bound IgG increased rapidly up to 6.15 μmol/mL of total lipids. Beyond this

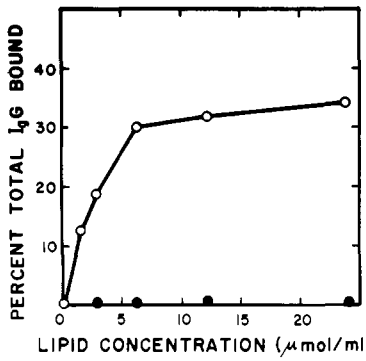

FIGURE 4: Effect of lipid to protein ratio on IgG–vesicle binding. [^{131}I]IgG (0.1 mg/mL) was sonicated with lipid mixtures (PC/chol/PG, 8.5:2.4:1.3 mole ratio) of increasing concentration for 30 min at power setting 1. Vesicle-bound IgG was separated from unbound IgG by chromatography on Sepharose 4B. IgG and lipids cosonicated (○) or sonicated separately and then mixed and incubated 30 min at 37 °C (●).

Table II: Binding of Various IgG's and Protein to Vesicles

protein	% total protein bound[a]
goat IgG	32
mouse IgG	35
human IgG	38
rabbit IgG	16
goat F(ab')$_2$	29
β-galactosidase	0

[a] Radioiodinated protein was sonicated with PC/chol/PG (8.5:2.4:1.3 μmol/mL) in PBS for 30 min at power setting 1. Concentrations of all proteins were 0.17 mg/mL, except for β-galactosidase which was 1 mg/mL. Vesicle-bound protein was separated from unbound protein by chromatography on Sepharose 4B.

concentration, only small amounts of further increase in bound IgG were observed (Figure 4). In routine experiments, we used 12.3 μmol/mL of PC/chol/PG and 0.1–0.5 mg/mL of IgG and sonicated for 30 min at power setting 1. Under these conditions 30–40% binding of the IgG was obtained.

While the major portion of this study dealt with goat IgG, binding of vesicles to IgG's from other species was also examined. Under identical sonication conditions, considerable amounts (16–38%) of IgG were bound to lipid vesicles by sonication; no significant difference was found from one species to another (Table II). Such affinity to bind lipid vesicles seems to be specific for IgG since another unrelated protein of similar molecular weight, i.e., β-galactosidase, did not bind to vesicles at all. Furthermore, the purified goat F(ab')$_2$ fragments also showed similar binding to vesicles when compared to IgG. Native or presonicated F(ab')$_2$ incubated with presonicated vesicles did not show any binding.

Stability of the Binding. The aggregated IgG–vesicle complexes were rather stable upon incubation at 37 °C. The complexes that were freshly prepared and isolated by using a preparatory Sepharose 4B column (see Materials and Methods section) showed a single peak at the void volume when refractionated on a small Sepharose 4B column (Figure 5a). After the complexes had been incubated at 37 °C for about 18 h in either PBS (Figure 5b) or 50% fetal calf serum (Figure 5c), about 20% of the originally bound IgG was released. The undissociated complexes were still aggregated since they eluted at the void volume of the column. Similar results were obtained if the complexes were not first separated from the unbound IgG and vesicles. Figure 5d summarizes the results of these experiments. Although vesicle-bound IgG

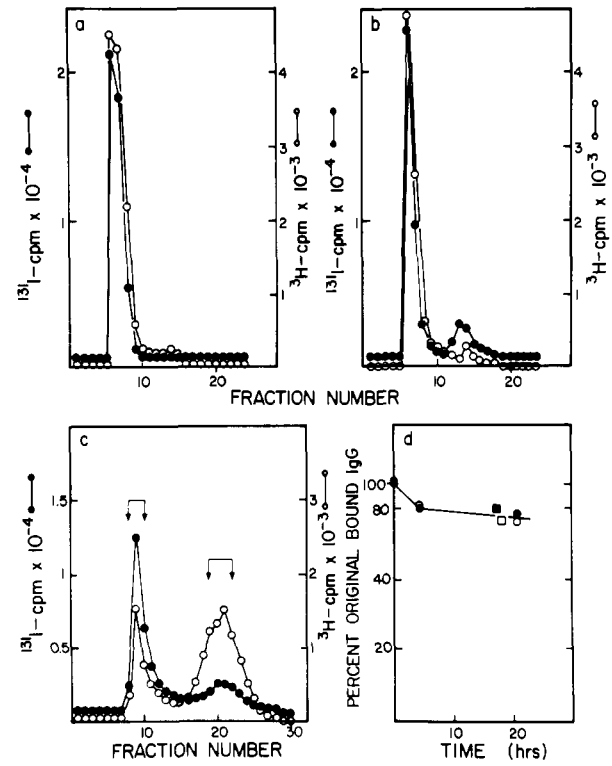

FIGURE 5: Stability of vesicle-bound IgG. [^{131}I]IgG (0.5 mg/mL) was sonicated with ^{3}H-labeled lipids (PC/chol/PG, 8.5:2.4:1.3 μmol/mL) at power setting 1 for 25 min. The bound IgG was first isolated by Sepharose 4B and incubated at 37 °C for 0 h (a), in PBS for 17.5 h (b) or in 50% fetal calf serum for 18.5 h (c). After incubation, a 0.5-mL sample was chromatographed on another Sepharose 4B column. The elution profiles of ^{3}H-cpm (○) and ^{131}I-cpm (●) are shown. Arrows in c indicated fractions pooled for lipid analysis by TLC. In d, the percent of IgG remained bound to vesicles was plotted against incubation time. Closed symbols represent incubations in PBS and open symbols in 50% fetal calf serum. Squares represent bound IgG was first separated from unbound IgG before incubation; circles represent no separation before incubation.

remained lipid associated in 50% fetal calf serum, some of the vesicle lipid was degraded during the incubation. Pooled fractions as indicated by arrows in Figure 5c were extracted by $CHCl_3$/MeOH (2:1) and the extracted lipids were analyzed by thin-layer chromatography. TLC showed that 85% of the ^{3}H radioactivity of fractions 19–22 was free fatty acids, whereas the lipids in the complexes (fractions 9–11) were not degraded. No degradation of lipids was found in IgG–vesicle preparations either freshly prepared or after 20 h of incubation at 37 °C in PBS. Also, no significant lipid degradation nor dissociation of the complexes was observed if the incubation was conducted at 4 °C for up to 20 h in either PBS or 50% fetal calf serum.

Position and Activity of Bound Antibody. It was important to determine if antibody bound to vesicles was still active and available for antigen binding. Immune IgG was labeled with ^{131}I and sonicated with lipids to generate vesicle-bound antibody. IgG bound to vesicles was separated from unbound IgG by Sepharose 4B. The IgG in these fractions was quantitated by the known specific activity of the original labeled material, and each fraction was tested for ability to bind protein antigen [^{125}I]gp70. Since gp70 is too large (molecular weight 70000) to enter the vesicles, only antibody on the outside should be able to bind it. Figure 6 shows that IgG bound to vesicles has the same capacity to bind antigen before and after treatment with 1.5% NP-40. Controls (not shown) indicated the following: (1) more than 90% of the ^{131}I-labeled immune IgG was precipitated in each case; (2)

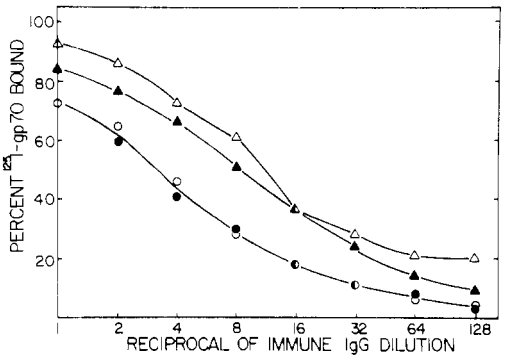

FIGURE 6: Antigen binding capacity of [^{131}I]IgG for [^{125}I]gp70. IgG (0.5 mg/mL) was sonicated with PC/chol/PG (8.5:2.4:1.3 μmol/mL) for 30 min at power setting 1. Vesicle-bound IgG was separated from unbound IgG by Sepharose 4B. Antigen binding curves of these preparations: vesicle bound IgG (●); vesicle bound IgG in 1.5% NP-40 (○); unbound IgG (▲); unbound IgG with presonicated vesicles (△).

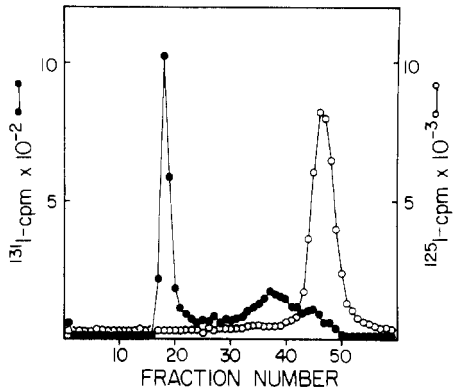

FIGURE 7: Sepharose 4B elution profile of vesicle-bound IgG in PBS containing 1% Triton X-100. [^{131}I]IgG (0.5 mg/mL) was sonicated with PC/chol/PG (8.5:2.4::1.3 μmol/mL) for 30 min at power setting 1. Bound IgG was separated from unbound IgG by Sepharose 4B. Bound IgG was then incubated 35 min at room temperature in 1% Triton X-100 before chromatography on another Sepharose 4B column. [^{125}I]αBGT (molecular weight 8000) was included as marker. Blue Dextran was eluted in fractions 17–19. ^{131}I-cpm (●) and ^{125}I-cpm (○) are shown.

NP-40 did not affect the antigen binding capacity of free immune IgG for [^{125}I]gp70; (3) normal IgG bound less than 5% of [^{125}I]gp70; and (4) sonication did not impair the antigen binding capacity of the IgG. Free IgG with or without added vesicles had an antigen binding capacity 1–1.5 times greater than bound IgG. Subsequent gel filtration of bound IgG in the presence of 1% Triton X-100 showed that bound IgG contained about 50% highly aggregated material which might have been denatured in the sonication step (Figure 7). This amount could account for the discrepancy in antigen binding capacity between bound and unbound IgG. It should be noted, however, that aggregation does not take place if IgG is sonicated without lipids present (see Figure 1a).

Discussion

The results presented in this report clearly demonstrate that goat IgG can be bound to phospholipid vesicles by sonication. Since IgG did not bind to preformed vesicles, cosonication was apparently required for binding. Two possible explanations for this finding are apparent. One is that some sort of conformational change of IgG has taken place during sonication. Such a change might be reversible in the absence of lipids since presonicated IgG does not bind to vesicles. In the presence of lipid vesicles, however, the altered conformation might enhance the affinity of IgG to bind to lipid membranes and result in stable binding. The fact that about 50% of bound IgG remains as high-molecular-weight aggregates in the presence of 1% Triton X-100 suggests such irreversible change in the conformaton of bound IgG (Figure 7). The extent of binding is critically dependent on the sonication conditions (power setting as well as length of sonication), indicating that the putative conformational change is a direct result of sonication. A second possible explanation of binding is that sonication produces transient perturbations in the vesicle bilayers and thus that the insertion of IgG into the bilayers is promoted. This hypothesis is supported by the facts that binding is dependent on the lipid/protein ratio and that an altered IgG, namely $F(ab')_2$, also binds to vesicles. While the actual mechanism(s) of binding cannot be determined at the present time, we have observed that the anionic phospholipids, but not cholesterol, can enhance the binding of IgG to vesicles. This suggests that ionic interactions between the lipids and the protein may be involved in the binding process.

Our findings are in apparent contrast to those of Weissmann et al. (1974), where they reported that human IgG can bind to large multilamellar anionic lipid vesicles without cosonication. The differences in the type of vesicles used and other experimental details make direct comparison between the two studies difficult. Binding of the heat aggregated human IgG to lipid vesicles involves the Fc region of the molecule (Weissman et al., 1974), which perturbs the vesicle membranes (Schieren et al., 1978). However, the Fc region of the goat IgG probably does not play an important role in the binding described here since $F(ab')_2$ fragments also bind to vesicles.

Sonication in the presence of lipids only partially inactivated the antigen binding capacity of the immune IgG since the vesicle-bound IgG retained 30–50% of the original activity. Since the same degree of antigen binding by the vesicle-bound antibody was observed before and after the vesicle membranes were lysed by 1.5% NP-40, and since the large protein antigen, gp70, could probably not penetrate through the vesicle membranes, the bound and active antibody appears to locate exclusively on the outer surface of the vesicles. In contrast, Gregoriadis & Neerunjun (1975) found that only 14.2–18.3% of rabbit IgG could be digested by Pronase when IgG was bound to vesicles by a similar, but not identical, sonication procedure. The vesicles were prepared by a brief 1-min sonication and were probably much larger in size than the ones reported here. It is therefore not surprising that they found a substantial amount of IgG trapped within the vesicles and unavailable to enzyme digestion.

The advantages of using immune IgG to modify the surfaces of lipid vesicles for targeting the vesicles to a specific tissue type include the following: (1) a substantial amount of IgG from various species can be bound to vesicle surfaces by simple sonication; (2) the bound IgG retains almost half of the original antigen binding capacity; and (3) the IgG–vesicle complexes are stable at 37 °C even in the presence of 50% serum. These advantages, however, are complicated by several potential drawbacks. The binding of IgG to vesicles causes extensive aggregation, resulting in a population of large particles. The systematic studies of Juliano & Stamp (1975) showed that large lipid vesicles were cleared from circulation of rats much faster ($t_{1/2}$ = 8 min) than small vesicles ($t_{1/2}$ = 80 min). The aggregated vesicle–IgG complexes may therefore be rapidly cleared from circulation, rendering the site-specific targeting of vesicles difficult. Furthermore, the heterogeneity of the IgG–vesicle aggregates may present problems in studying the mechanism of interaction between cells and these vesicles (vesicle–cell fusion, endocytosis, adsorption and lipid/protein exchange; for review, see Pagano & Weinstein, 1978). It is

important to determine the subcellular compartment(s) (cytoplams, lysosomes, or other organelles) to which these types of vesicles can deliver their contents. We are currently evaluating these problems.

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Phospholipid Lateral Phase Separation and the Partition of *cis*-Parinaric Acid and *trans*-Parinaric Acid among Aqueous, Solid Lipid, and Fluid Lipid Phases†

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ABSTRACT: The partition of *cis*-parinaric acid (9,11,13,15-*cis*,*trans*,*trans*,*cis*-octadecatetraenoic acid, *cis*-PnA) and *trans*-parinaric acid (9,11,13,15-*all-trans*-octadecatetraenoic acid, *trans*-PnA) among aqueous, solid lipid, and fluid lipid phases has been measured by three spectroscopic parameters: absorption spectral shifts, fluorescence quantum yield, and fluorescence polarization. The solid lipid was dipalmitoylphosphatidylcholine (DPPC); the fluid lipid was palmitoyldocosahexaenoylphosphatidylcholine (PDPC). Mole fraction partition coefficients between lipid and water were determined by absorption spectroscopy to be, for *cis*-PnA, 5.3×10^5 with solid lipid and 9×10^5 with fluid lipid and, for *trans*-PnA, 5×10^6 with solid lipid and 1.7×10^6 with fluid lipid. Ratios of the solid to the fluid partition coefficients ($K_p^{s/f}$) are 0.6 ± 0.2 for *cis*-PnA and 3 ± 1 for *trans*-PnA. A phase diagram for codispersions of DPPC and PDPC has been constructed from the measurements of the temperature dependence of the fluorescence quantum yield and polarization of *cis*-PnA and *trans*-PnA and their methyl ester derivatives. A simple analysis based on the phase diagram and fluorescence data allows additional calculations of $K_p^{s/f}$'s which are determined to be 0.7 ± 0.2 for the cis probes and 4 ± 1 for the trans probes. The relative preference of *trans*-PnA for solid phase lipids and its enhanced quantum yield in solid phase lipids make it sensitive to a few percent solid. The trans probes provide evidence that structural order may persist in dispersions of these phospholipids 10 °C or more above their transition temperature. It is concluded that measurements of PnA fluorescence polarization vs. temperature are better suited than measurements of quantum yield vs. temperature for determining phospholipid phase separation.

Fluorescent probe molecules continue to contribute to the elucidation of membrane microstructure.[1] This work further develops *cis*-parinaric acid (*cis*-PnA,[2] 9,11,13,15-*cis*,*trans*,*trans*,*cis*-octadecatetraenoic acid) and *trans*-parinaric acid (*trans*-PnA, 9,11,13,15-*all-trans*-octadecatetraenoic acid) as fluorescent probes for biological membranes. In previous publications we have characterized the fluorescence response of these molecules to phospholipid phase transitions and lateral

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[1] A recent review (Lee, 1977) contains leading references to the application of spectroscopic and other physical methods to phospholipid phase diagrams.

[2] Abbreviations used: *cis*-PnA, *cis*-parinaric acid; BHT, 2,6-di-*tert*-butyl-4-methylphenol; DPPC, dipalmitoylphosphatidylcholine; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid; ME, methyl ester; *P*, fluorescence polarization ($I_{\parallel}/I_{\perp}$); PC, phosphatidylcholine; PDPC, 1-palmitoyl-2-docosahexaenoylphosphatidylcholine; *Q*, fluorescence quantum yield; TLC, thin-layer chromatography; *trans*-PnA, *trans*-parinaric acid.

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