Biochimica et Biophysica Acta, 684 (1982) 187–194
Elsevier Biomedical Press

BBA 71024

SPECIFIC REACTIVITY OF LIPID VESICLES CONJUGATED WITH ORIENTED ANTI-LACTOSE ANTIBODY FRAGMENTS

DEEPALI SINHA and FRED KARUSH

Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 (U.S.A.)

(Received June 3rd, 1981)
(Revised manuscript received September 28th, 1981)

Key words: Liposome targeting; Fluorescence quenching; Membrane-conjugated antibody

The method previously described (Sinha, D. and Karush, F. (1979) Biochem. Biophys. Res. Commun. 90, 554–560) for the oriented attachment of immunoglobulins to lipid vesicles has been used to confer specific reactivity on liposomes by their conjugation with anti-lactose Fab′ fragments derived from rabbit IgG antibody. It is estimated that one-third of the Fab′ fragments was irreversibly attached to liposomal membrane, resulting in a membrane concentration of 2 mmol of Fab′ per mol of total lipid. The specific reactivity of the modified liposomes was demonstrated by agglutination with a multivalent, lactose-containing diheteroglycan. The availability of virtually all of the binding sites of the attached antibody for reaction with ligand was established by a fluorescence quenching titration with *N*-(*N*$^{\varepsilon}$-Dnp-L-lysyl)-*p*-aminophenyl-β-lactoside. An intrinsic association constant of $8.9 \cdot 10^6$ M^{-1} was found for the attached Fab′ compared to a value of $2.8 \cdot 10^6$ M^{-1} for free anti-lactose Fab′. In addition the maximum values for the quenching by bound ligand of the fluorescence of free and attached antibody were the same. It can be concluded that the chemical procedures used to effect attachment of the antibody to the lipid vesicles allow retention of the original structure of the antibody site and its accessibility to external components.

Introduction

The potential utility of specific antibody for the targeting of liposomes has been widely recognized in recent years and has inspired a variety of studies (e.g. Refs. 1–3). This prospect has acquired enhanced significance as a result of the availability of homogeneous antibodies through the use of the hybridoma technology [4–6]. One of the requirements for this use of antibody is its stable attachment to the vesicle membrane in a form and amount which will permit specific and high-affinity (i.e. multivalent) binding to target cells. Several methods for the attachment of immunoglobulin and other proteins to lipid vesicle membranes have been described [2,3,7–10]. They may be limited in their applicability, however, because of low efficiency of conjugation and/or because the specific reactivity of the protein is reduced either for steric reasons or by virtue of chemical modification.

We have recently developed a method for the attachment of immunoglobulin to lipid membranes which involves the conjugation of a hydrophobic anchor to a specific site on the protein, in particular, the SH group generated by the reductive cleavage of the interheavy chain disulfide bond [11]. As a result the subsequent insertion of the anchor into the membrane is expected to yield an orientation of the protein such that the combining sites of the antibody remain accessible to ligands in solution. An alternative utilization of the SH

Abbreviations: t-Boc, *t*-butoxycarbonyl-; Dnp-, dinitrophenyl-; Dns-, dansyl-.

group for the site-specific attachment of a monovalent antibody fragment to liposomal membrane was subsequently described by Martin et al. [12]. In this report we describe the procedure for the preparation of the conjugated derivative of the Fab′ fragment derived from rabbit anti-lactose antibody. We also demonstrate that the specific reactivity of the antibody remains unchanged after attachment to the lipid vesicles. This conclusion was based on the measurement by the fluorescence quenching technique of the affinity for a lactosyl ligand in its interaction with Fab′ in solution and with membrane-attached Fab′. A summary of the results described in this report has been presented previously [13].

Methods

Reagents. Dipalmitoylphosphatidylethanolamine (DPPE), dicyclohexylcarbodiimide, dioleoylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DPMC), diphenylhexatriene and cholesterol were purchased from Sigma (U.S.A.). [^{3}H]acetic anhydride (500 mCi/mmol) was obtained from Amersham and [^{3}H]cholic acid (16 Ci/mmol) from New England Nuclear.

Preparation of [^3H]F(ab′)$_2$. Anti-lactose antibody was elicited in female white New Zealand rabbits immunized with *p*-aminophenyl-β-lactoside conjugated to bovine γ-globulin [14]. The specific antibody was purified using a lactose affinity column and fractionated on a Sephadex G-200 column [14]. The F(ab′)$_2$ fragment of the IgG fraction was prepared by peptide digestion [15]. The antibody (30–40 mg) was dissolved in 1 ml of 0.1 M acetate buffer, pH 4.1 containing 0.3–0.4 mg pepsin (Calbiochem). The reaction mixture was incubated for 16–17 h at 37°C and then chromatographed on a Sephadex G-200 column (55 × 2.6 cm) with 0.02 M NH_4HCO_3. The fractions containing F(ab′)$_2$ were pooled and lyophilized. To label the protein with tritium, the lyophilized material (20–30 mg) was dissolved in 1 ml of 0.5 M $NaHCO_3$ and cooled in an ice bath. Five portions of the acetylating reagent [^{3}H]acetic anhydride, each containing 1 mCi in 20 μl of solvent (50% benzene, 50% dioxane), were added over a period of one hour. Excess labeling reagent was removed by gel filtration and dialysis.

Synthesis of the alkylating agent N-(N$^\alpha$-iodoacetyl, N$^\epsilon$-dansyl-lysyl)phosphatidylethanolamine (iodo-ADLPE). The preparation of this compound has been described in a previous report [11]. Briefly, N$^\alpha$-t-Boc, N$^\epsilon$-dansyllysine was reacted with dipalmitoylphosphatidylethanolamine in the presence of dicyclohexylcarbodiimide (DCCI). The α-amino group of the lysine moiety of the resulting product was then deblocked with trifluoroacetic acid yielding N-(N$^\epsilon$-dansyl-lysyl)phosphatidylethanolamine (DLPE). The product was crystallised from ethyl acetate and further purified by passage through a silicic acid column using $CHCl_3/CH_3OH/H_2O$ (80 : 19 : 1, v/v) (solvent A). The iodoacetyl derivative of DLPE was prepared by reacting the latter with iodoacetic acid in the presence of DCCI. Excess iodoacetic acid was removed by repeated recrystallization from ethyl acetate. The product contained some unreacted DLPE as observed from TLC developed in solvent A but was used without further purification [11].

Reduction of [^3H]F(ab′)$_2$ and alkylation with iodo-ADLPE. Conjugation of the hydrophobic anchor ADLPE to the Fab′ fragment has been done by a modification of our previous procedure [11]. [^{3}H]F(ab′)$_2$ was reduced with 10 mM mercaptoethanol in 0.1 M Tris-HCl, pH 8.4 at room temperature for 2 h under N_2. This treatment provides a high yield of the Fab′ fragment in which the L-H disulfide bond is intact and a reactive SH group is located at the C-terminal end of the heavy chain fragment. After adjustment of the pH to 3.5 with glacial acetic acid the reaction mixture was chromatographed on a Sephadex G-25 column, equilibrated with 0.01 M acetic acid containing 2 mM EDTA, to remove excess reducing agent. After removal of mercaptoethanol the reduced protein was alkylated with iodo-ADLPE in the presence of 0.2% Nonidet P-40 at pH 8.0 under N_2 at room temperature for 24 h. In this reaction a ratio of 4 mol of iodo-ADLPE/mol of Fab′ was used and the Nonidet P-40 served to keep iodo-ADLPE in solution. Formation of Fab′-ADLPE was confirmed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using a 4.4% gel in 0.1 M phosphate buffer (pH 7.2), 0.1% in SDS. The gel showed a major fluorescent band at the position of heavy chain (H) derived from

normal rabbit IgG as expected for Fab'-ADLPE. A minor fluorescent band was also seen near the light chain (mol. wt. approx. 25000) region indicating reduction of some of the H-L disulfide bonds and their subsequent alkylation with iodo-ADLPE. Free iodo-ADLPE showed up as a fluorescent band that moved ahead of the bromophenol blue tracking dye. The gels, after staining with Coomassie brilliant blue, were scanned with an ISCO model UA-5 Absorbance Monitor equipped with a model 1310 gel scanner.

Purification of [^{3}H]Fab'-ADLPE. The reaction mixture (10 – 12 ml), after reduction and alkylation, was passed through a DEAE-Sephadex column (20 ml) equilibrated with 0.01 M Tris-acetate at pH 8.0. The column retained unreacted [^{3}H]F(ab')$_2$, the protein fragments conjugated with ADLPE as well as free iodo-ADLPE but not Nonidet P-40. This separation was indicated by the absence of any dansyl fluorescence or radioactivity in the eluates although the effluent showed absorbance at 280 nm. The column was extensively washed with the same buffer and then eluted with 0.72 M NaCl in 0.01 M Tris-acetate, pH 8.0. The eluates showed the presence of radioactivity and absorbance at 280 nm but no dansyl fluorescence, indicating the elution of [^{3}H]F(ab')$_2$. Elution was continued with 0.72 M NaCl in 0.01 M Tris-acetate, pH 8.0 containing 0.25% sodium cholate. The material eluted with this solvent showed 280 nm absorbance, radioactivity and dansyl fluorescence. The ratio of Fab': dansyl was 1.0, calculated from the absorbance at 280 nm for the protein and at 340 nm for the dansyl group with correction for the dansyl absorption at 280 nm. A value of 4600 $M^{-1} \cdot cm^{-1}$ was used for the dansyl extinction coefficient at 340 nm [16]. The eluates were pooled, concentrated and extensively dialysed vs. 0.1 M Tris-HCl, pH 8.0 with three changes of 2.5 liters of buffer each time over a period of 5 days to remove most of the cholate from the protein. A control experiment done with unlabeled Fab'-ADLPE and [^{3}H]cholate indicated that the residual cholate did not exceed 0.002%. Two preparations of [^{3}H]Fab'-ADLPE were made, one starting with rabbit anti-lactose IgG (anti-lactose [^{3}H]Fab'-ADLPE) and another starting with normal rabbit IgG (normal [^{3}H]Fab'-ADLPE).

Preparation of lipid vesicles. A thin film of lipid (either dioleoylphosphatidylcholine (DOPC) or dimyristoylphosphatidylcholine (DPMC) was made by evaporating its chloroform solution in a glass test tube. The lipid was suspended in 0.1 M Tris-HCl, pH 8.0 at a concentration of 1 mg lipid/ml. The suspension was mixed by vortexing for several minutes and then sonicated for 3–5 min with the microtip of a Branson sonicator (LS75) at room temperature in the case of DML and at 4–5°C in the case of DOPC. The sonicated material was centrifuged at 14000 rev./min (23500 × g) and the clear supernatant was used in the experiment. In cases where a mixture of phosphatidylcholine and cholesterol was used the chloroform solutions of the two were mixed together before evaporating off the solvent.

Incorporation of normal [^{3}H]Fab'-ADLPE and anti-lactose [^{3}H]Fab'-ADLPE into liposomal membrane. Vesicles at a lipid concentration of 1 mg/ml were incubated with the conjugated protein (5 μM) for 24 h at room temperature with gentle agitation. The mixture (0.5 ml) was then subjected to gel filtration on Bio Gel A-5m (0.8 × 54 cm) equilibrated with 0.1 M Tris-acetate, pH 8.0. Fractions of 0.4 ml were collected and the elution pattern of the protein was obtained by counting an aliquot of each fraction in a liquid scintillation counter. Relative concentrations of the lipid in the eluates were measured by adding 0.25 ml of a suspension of diphenylhexatriene (100 μM) to 0.2 ml of each fraction. The mixture was incubated for 2 h at room temperature and the fluorescence emission of diphenylhexatriene measured at 420 nm (excitation at 357 nm) using a Perkin-Elmer 512 Spectrofluorimeter. The fluorescence emission of diphenylhexatriene is totally quenched in the aqueous medium but reappears as it enters the lipid vesicle membrane [17]. In the range of concentrations used in our experiment the fluorescence intensity of diphenylhexatriene was found to be linearly related to the concentration of the lipid.

Agglutination of modified liposomes. DOPC vesicles, after being incubated with either normal Fab'-ADLPE or anti-lactose Fab'-ADLPE and purified by gel filtration as described above, were treated with either normal rabbit serum (diluted 1:100) or rabbit anti-dansyl anti-serum (diluted 1:100) [14], or with a diheteroglycan containing

multiple lactosyl groups, isolated from the cell wall of *Streptococcus faecalis* (strain N) [18], at a concentration of 1 μg/ml.

Retention of vesicles with attached anti-lactose Fab'-ADLPE on a lactose affinity column. Lipid vesicles made from dioleoylphosphatidylcholine and cholesterol (90:10, w/w) were incubated with either normal [^{3}H]Fab'-ADLPE or anti-lactose [^{3}H]Fab'-ADLPE for 24 h at room temperature. These preparations were then centrifuged at 80000 $\times g$ in the Beckman Airfuge for 40 min and the supernatants removed. The pellets were resuspended and centrifuged again two times to assure adequate removal of the free protein conjugate. The washed pellet was resuspended in phosphate buffered saline (0.15 M NaCl, 0.02 M phosphate), pH 7.4 and passed through a lactose affinity column. The protein in the fractions was measured by counting the radioactivity and the lipid by measuring the fluorescence of diphenylhexatriene in the membrane as described before. In order to elute all of the protein it was necessary to treat the top of the column with either 0.1% SDS or 0.25% cholate containing 0.3 M lactose for 30 min and then continue elution with this solution.

Affinity measurement by fluorescence quenching. Vesicles prepared with DOPC and cholesterol (90:10, w/w)) and attached with either normal [^{3}H]Fab'-ADLPE or anti-lactose [^{3}H]Fab'-ADLPE were prepared free of any protein in solution by the procedure described above. The former was used as a control for dilution and non-specific quenching in the fluorescence quenching titration. The protein fluorescence at 25°C was measured as a function of the amount of a Dnp-conjugated lactosyl ligand (EK), *N*-(*N*$^\epsilon$-Dnp-L-lysyl)-*p*-aminophenyl-β-lactoside [19], in the titration solution. The Perkin-Elmer 512 Spectrofluorimeter was used in the ratio mode with diffuse plates in the reference cell holder, The fluorescence was measured at 345 nm with excitation at 280 nm. Since there was an appreciable contribution to the signal at 345 nm due to scattering by the vesicles, a further correction was made by measuring the apparent 345 nm emission of a vesicle preparation at the same lipid concentration and subjected to the same procedures of centrifugation and washing as done with the samples. The fluorescence titration was also carried out with anti-lactose Fab' (prepared by alkylating with iodoacetamide instead of iodo-ADLPE) using normal IgG to correct for dilution and attenuation. To 0.4 ml of a solution containing about 1 μM Fab' in 0.15 M NaCl, 0.02 M phosphate, pH 7.4 were added increments of 100 μM solution of the EK compound to a total addition of 5 to 20 μl. The maximum quenching (Q_{max}) was obtained by extrapolation of the measured quenching. Binding constants were calculated from the fluorescence data [20] in conjunction with the Sips analysis [21] using a program developed by Dr. Robert Luedtke and computations performed on a Commodore PET 2001 microcomputer.

Results

Preparation of [^{3}H]Fab'-ADLPE.

The reduction of [^{3}H]Fab$'_2$ and the alkylation of the free sulfhydryl group of the product provide a high yield of the desired product. This result is evident in Fig. 1 (Panel A) where the peak under the H chain marker represents the [^{3}H[Fab' fragment. There are also present in the reaction mixture unreacted F(ab')$_2$ and fluorescent fragments of a molecular weight similar to that of L chain. The latter undoubtedly arise from reductive cleavage of the L-H disulfide bond.

The purification of the desired product was achieved by the differential elution from a DEAE-Sephadex column. The hydrophobic group (ADLPE) attached to the Fab' fragment increased its retention relative to that of F(ab')$_2$ and led to the use of cholate for the elution of the Fab' fragment (Fig. 2). The effectiveness of the procedure is seen in the scan of the SDS polyacrylamide gel electrophoresis pattern of the purified [^{3}H]Fab' (Panel B of Fig. 1). The only detectable contaminant, present as a minor component, is the unreacted F(ab')$_2$.

Association of Fab'-ADLPE with vesicles.

The attachment of the modified fragment to the vesicles is demonstrated by gel filtration with Bio Gel A-5m (Fig. 3). This procedure provides a complete separation of the bound protein from the free protein. It is estimated that about one-third of the added protein was attached to the vesicles. This attachment is virtually irreversible in aqueous

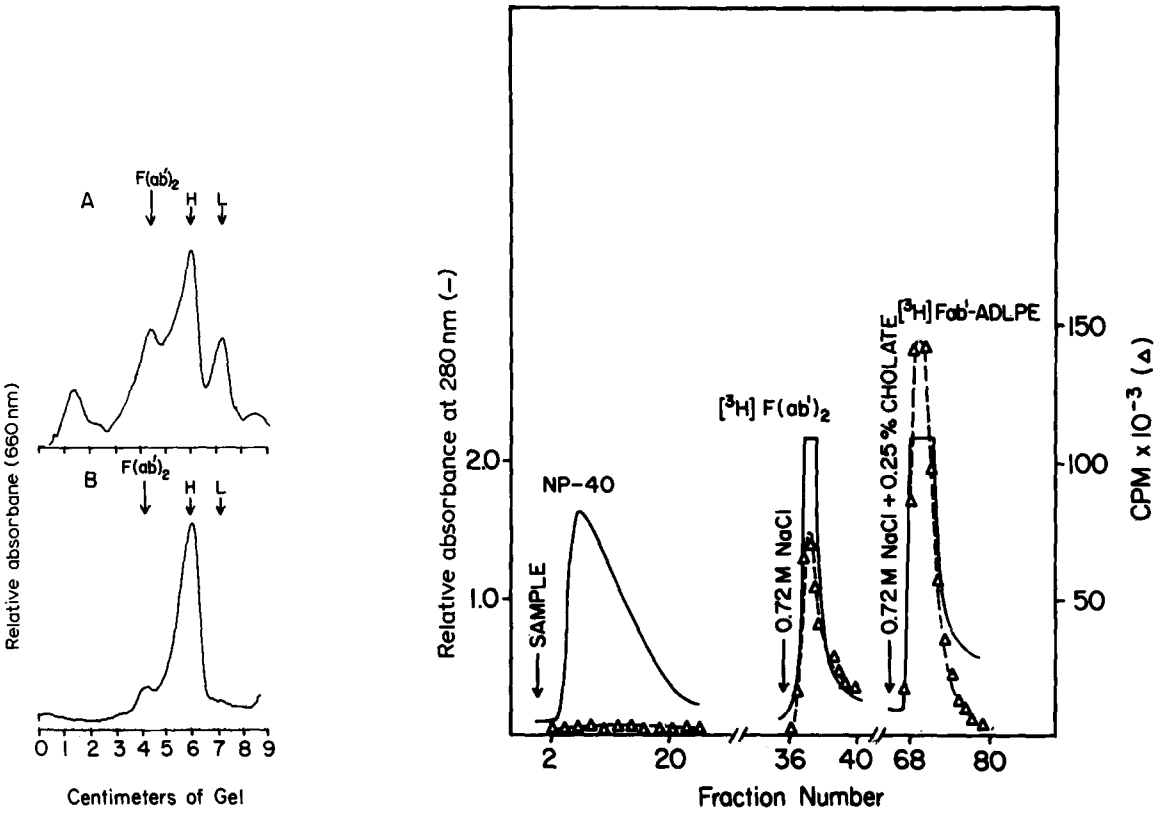


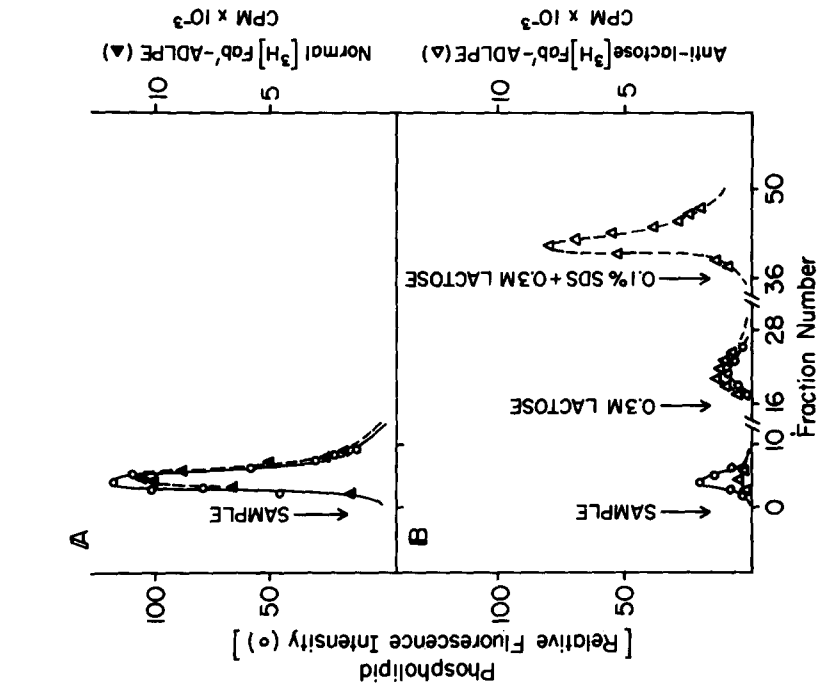
Fig. 1. SDS polyacrylamide gel electrophoresis scans of (A) the reaction mixture after reduction of $F(ab')_2$ and its subsequent alkylation with iodo-ADLPE; and (B) purified Fab'-ADLPE preparation. The positions of $F(ab')_2$ heavy (H) and light (L) chains are shown by arrows.

Fig. 2. Purification of [^{3}H]Fab'-ADLPE on a DEAE-Sephadex column. The reaction mixture, after reduction of $F(ab')_2$ followed by alkylation with iodo-ADLPE, was passed through a DEAE-Sephadex column (20 ml) equilibrated with 0.01 M Tris-acetate at pH 8.0. The column was washed extensively with the same buffer to remove Nonidet P-40 (NP-40). Elution of unreacted [^{3}H]$F(ab')_2$ was effected with 0.72 M NaCl solution and that of [^{3}H]Fab'-ADLPE with 0.72 M NaCl in 0.25% sodium cholate solution. Unreacted free iodo-ADLPE and its degradation products remained tightly bound on top of the column.

solvent as is evident from Panel C of Fig. 3 and confirmed by a second gel filtration (not shown). Incubation of vesicles with the Fab' fragment alkylated with iodoacetamide led to no significant attachment of the protein. This observation is similar to the results with the λ chain previously reported [11]. The integrity of the vesicles after incubation with the modified fragment was established by the incorporation of 0.1 M carboxyfluorescein [22] during the preparation of the vesicles. Analysis by gel filtration of such vesicles both before and after incubation with the protein conjugate showed equivalent retention of the incorporated marker, namely, 85–90%.

Specific agglutination

The agglutination experiments with anti-dansyl anti-serum and with the diheteroglycan containing multiple lactosyl groups yielded the anticipated results. Thus, the vesicles with either normal or anti-lactose Fab'-ADLPE attached to them were agglutinated with anti-dansyl antibodies whereas normal rabbit serum was ineffective. On the other hand with the diheteroglycan only the vesicle preparation with attached anti-lactose Fab'-ADLPE was agglutinated. This agglutination was inhibited with 0.3 M lactose. In addition vesicles without either protein conjugate were not agglutinated with any reagent.



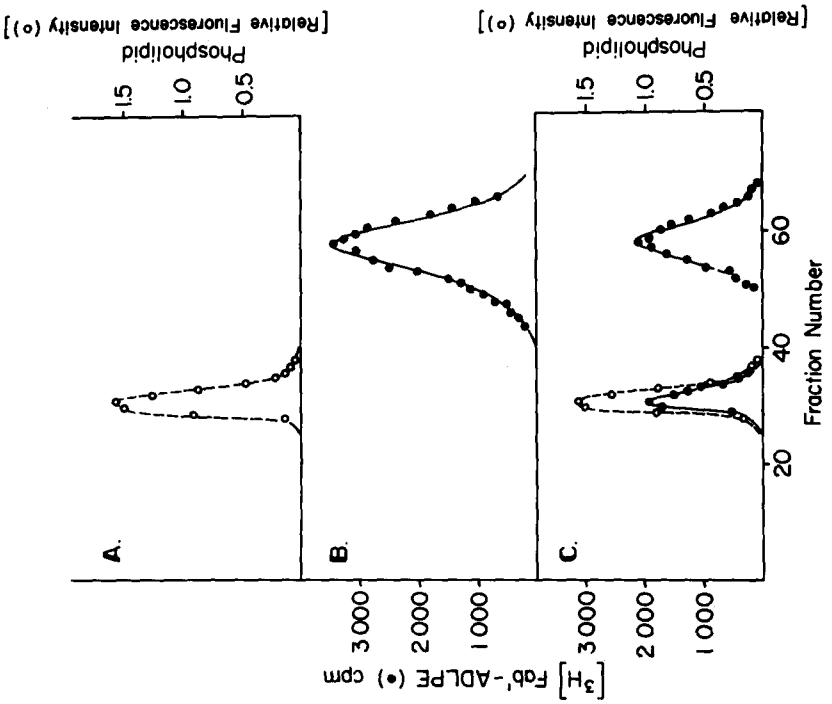


Fig. 3. The retention of [^{3}H]Fab'-ADLPE by vesicles after incubation. Samples in 0.5 ml of 0.1 M Tris-acetate buffer, pH 8.0 and 2 mM EDTA contained (A) 1 mM DOPC (liposomal), (B) 5 μM [^{3}H]Fab'-ADLPE and (C) 5 μM [^{3}H]Fab'-ADLPE and 1 mM phospholipid (liposomal). Samples were incubated at room temperature for 24 h before gel filtration. The samples were applied to a column (0.8 × 54 cm) of Bio-Gel A-5m in 0.1 M Tris-acetate buffer, pH 8.0 and 0.02% NaN_3 and fractions of 0.4 ml collected. The elution patterns of the proteins were obtained by assay of radioactivity and those of the phospholipid by fluorescence (see text).

Fig. 4. Binding of DOPC vesicles with attached anti-lactose Fab'-ADLPE to Lac-Sepharose affinity column. A sample in 0.5 ml of 0.15 M NaCl, 0.02 M phosphate, pH 7.4 containing 1 mM lipid (liposomal) with bound (A) normal [^{3}H]Fab'-ADLPE (2.6 μM) or (B) anti-lactose [^{3}H]Fab'-ADLPE (2.6 μM) was passed through a Lac-Sepharose affinity column (3 ml bed volume) equilibrated with the same solvent and fractions of 0.5 ml collected.

Specific binding to a lactose affinity column

The specific binding of vesicles with attached anti-lactose [^{3}H]Fab'-ADLPE has been demonstrated with a Lac-Sepharose affinity column (Fig. 4). The absence of non-specific binding was established with normal [^{3}H]Fab'-ADLPE (Panel A, Fig. 4) which emerged in the void volume in association with the vesicles. With the anti-lactose fragment (Panel B, Fig. 4) only 8% of the lipid and 4% of the protein emerged in the void volume. Elution with 0.3 M lactose yielded an additional 10% of protein and 10% of lipid. The bulk of the material required the use of a detergent. The elution of a portion of the vesicles with 0.3 M lactose probably reflects the heterogeneous nature of the antibody population with respect to its intrinsic affinity for lactose. The need for detergent to elute the bulk of the vesicles undoubtedly arises from their multivalent attachment to the affinity column.

Intrinsic affinity of attached anti-lactose Fab'-ADLPE

The fluorescence titrations of the free and attached anti-lactose fragments are shown in Fig. 5. The calculated values of the average intrinsic association constant (K) and of Q_{max} are $2.8 \cdot 10^6$ M^{-1} and 90% for the free anti-lactose Fab' and $8.9 \cdot 10^6$ M^{-1} and 90% for the attached anti-lactose Fab'-ADLPE, respectively.

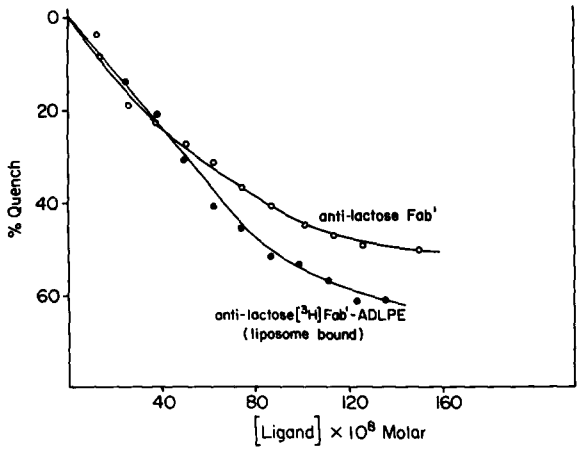


Fig. 5. Quenching of fluorescence of free anti-lactose [^{3}H]Fab' (○) and of vesicle-bound anti-lactose [^{3}H]Fab'-ADLPE (●) by titration with EK-compound. The excitation wavelength was 280 nm and emission was measured at 345 nm. The fluorescent emission in the case of vesicle-bound anti-lactose-[^{3}H]Fab'-ADLPE was corrected for the contribution due to scattering from the vesicles by measuring the apparent 345 nm emission of a vesicle preparation at the same lipid concentration as used for the sample.

Discussion

The main thrust of this study is the demonstration of a procedure which allows the oriented attachment of an antibody fragment to liposomal membrane with retention of its specific reactivity and without impairment of its intrinsic affinity. Since the composition of this system is known and the mol fraction of the antibody in the membrane, as well as its bulk concentration, can be calculated, the system allows quantitative kinetic and equilibrium measurements of the interaction of the antibody with ligands in the bulk phase. The fluorescence titrations described in this report provide an example of the utility of model membrane vesicles with attached antibody to explore its specific reactivity. With judicious selection of components and conditions such a system can provide useful models for the interactions of B-cell and T-cell receptors. The utilization of monoclonal antibody or its fragments for attachment to the membrane will greatly simplify the physical-chemical interpretation of the proposed measurements. Finally, it is of interest to note that the procedure described here for the attachment of immunoglobulin to liposomal membranes also has potential applicability to the membranes of living cells.

The extent of attacment of the Fab' fragment to the liposomal membrane corresponds to approx. 2 mmol of Fab' per mol of total lipid. This value is comparable to the highest values reported for other systems, namely, the covalent attachment of horseradish peroxidase [3] and the covalent attachment of immunoglobulins by the reaction of the aldehyde group of a membrane component with amino groups of the protein [7]. Our procedure differs significantly from these by virtue of the defined point of attachment of the hydrophobic anchor to the antibody fragment.

The fluorescence quenching results allow us to conclude that there has been no loss of intrinsic affinity of the antibody binding sites by virtue of the chemical manipulations involved in the attach-

ment procedure. This conclusion is drawn from the fact that the value of the association constant K) for the attached Fab' ($8.9 \cdot 10^6$ M^{-1}) is not less than that for the free Fab' ($2.8 \cdot 10^6$ M^{-1}). The significance of the finding of an increased binding constant is not clear but it may arise from the restricted rotational freedom of the attached Fab' moiety.

The retention of the activity of all of the combining sites after attachment is indicated by the equal values for Q_{max}. Although the determination of Q_{max} provided only approximate values because of the heterogeneity of the antibody population, a substantial loss of sites would have yielded a lower value for the attached Fab' than that for the free Fab' because of the consistent manner by which Q_{max} was calculated. It should also be emphasized that the values of K are relatively insensitive to the assigned values of Q_{max}.

Finally, it is evident that the desired capability of multivalent interaction has been acquired by the liposomes with the attached anti-lactose Fab' fragments. This conclusion is drawn from the inability to elute these liposomes from the Lac-Sepharose affinity column with 0.3 M lactose, a concentration which is sufficient to elute anti-lactose IgG antibody from such a column [14].

Acknowledgments

We are indebted to Dr. Robert Luedtke for his contribution of the anti-lactose and anti-dansyl antisera and for the use of his computer program. We are also grateful to Sally Karush for her skillful preparation of the manuscript. This study was supported by USPHS grants AI-09492 (research grant to Fred Karush), CA-09140 (traineeship to Deepali Sinha), AI-14012 (research career award to Fred Karush) and a research grant from the American Cancer Society (IM-259).

References

1 Bankert, R.B., Mayers, G.L. and Pressman, D. (1979) J. Immunol. 123, 2466–2474
2 Huang, A., Huang, L. and Kennel, S.J. (1980) J. Biol. Chem. 255, 8015–8018
3 Heath, T.D., Robertson, D., Birbeck, M.S.C. and Davies, A.J.S. (1980) Biochim. Biophys. Acta 599, 42–62
4 Köhler, G. and Milstein, C. (1976) Eur. J. Immunol. 6, 511–519
5 Potter, M. (1972) Physiol. Rev. 52, 631–719
6 Kennett, R.H., Denis, K.A., Tung, A.S. and Klinman, N.R. (1978) Curr. Top. Microbiol. Immunol. 81, 77–91
7 Heath, T.D., Macher, B.A. and Papahadjopoulos, D. (1981) Biochim. Biophys. Acta 640, 66–81
8 Leserman, L.D., Barbet, J., Kourilsky, F. and Weinstein, J.N. (1980) Nature 288, 602–604
9 Torchilin, V.P., Khaw, B.A., Smirnov, V.N. and Haber, E. (1979) Biochem. Biophys. Res. Commun. 89, 1114–1119
10 Wickner, W.T. (1977) Biochemistry 16, 254–258
11 Sinha, D. and Karush, F. (1979) Biochem. Biophys. Res. Commun. 90, 554–560
12 Martin, F.J., Hubbell, W.L. and Papahadjopoulos, D. (1981) Biochemistry 20, 4229–4238
13 Sinha, D. and Karush, F. (1980) Fed. Proc. 39, 1761x
14 Luedtke, R., Owen, C.S. and Karush, F. (1980) Biochemistry 19, 1132–1192
15 Fanger, M.W., Hart, D.A., Wells, J.V. and Nisonoff, A. (1970) J. Immunol. 105, 1484–1492
16 Stryer, L. (1965) J. Mol. Biol. 13, 482–495
17 Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652–2657
18 Pazur, J.H., Cepure, A. and Kane, J. (1973) J. Biol. Chem. 248, 279–284
19 Gopalakrishnan, P.V. and Karush, F. (1975) J. Immunol. 114, 1359–1362
20 Eisen, H.N. and McGuigan, J.E. (1971) in Methods in Immunology and Immunochemistry (Williams, C.A. and Chase, M.W., eds.), vol. 3, pp. 395–406, Academic Press, New York
21 Karush, F. (1962) in Advances in Immunology (Dixon, F.J., Jr. and Humphrey, J.H., eds.), vol. 2, pp. 1–40, Academic Press, New York
22 Weinstein, J.N., Yoshikami, S., Henkart, P. Blumenthal, R. and Hagins, W.A. (1977) Science 195, 489–492