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Intervesicular exchange of lipids with weak acid and weak base characteristics: influence of transmembrane pH gradients

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Transmembrane pH gradients have previously been shown to induce an asymmetric transmembrane distribution of simple lipids that exhibit weak acid or basic characteristics (Hope, M.J. and Cullis, P.R. (1987) J. Biol. Chem. 262, 4360–4366). In the present study we have examined the influence of proton gradients on the inter-vesicular exchange of stearylamine and oleic acid. We show that vesicles containing stearylamine immediately aggregate with vesicles containing phosphatidylserine and that disaggregation occurs subsequently as stearylamine equilibrates between the two vesicle populations. Despite visible flocculation during the aggregation phase, vesicle integrity is maintained. Stearylamine is the only lipid to exchange, fusion does not occur and vesicles are able to maintain a proton gradient. When stearylamine is sequestered to the inner monolayer in response to a transmembrane pH gradient (inside acidic) aggregation is not observed and diffusion of stearylamine to acceptor vesicles is greatly reduced. The ability of ΔpH-dependent lipid asymmetry to modulate lipid exchange is also demonstrated for fatty acids. Oleic acid can be induced to transfer from one population of vesicles to another by maintaining a basic interior pH in the acceptor vesicles. Moreover, it is shown that the same acceptor vesicles are capable of depleting serum albumin of bound fatty acid. These results are discussed with respect to the mechanism and modulation of lipid flow between membranes both in vitro and in vivo.

Introduction

Lipid exchange is an important physiological process. The diffusion of fatty acids between micelles, fatty acid binding proteins and cell membranes represent fundamental steps in the metabolism and biosynthesis of lipids. Fatty acid exchange between membranes or from membranes to fatty acid-binding proteins such as albumin requires that a lipid monomer leave the bilayer and diffuse through the aqueous medium to an acceptor site. The rate-limiting step in this process is release of monomers from the membrane [1–4]. In the case of long chain phospholipids [1,5] and glycolipids [6] spontaneous exchange is slow in the absence of specific transfer proteins. However, for fatty acids this process is rapid [4,7] even in the absence of fatty acid binding proteins, which represent a significant proportion of the cytosolic protein mass of many cells [8,9].

The present work originated from an observation that when vesicles containing stearylamine were mixed with vesicles containing phosphatidylserine, a rapid aggregation occurred resulting in visible flocculation of the sample. However, the vesicle mixture reverted to its original appearance within minutes. This could be explained if stearylamine could exchange rapidly between vesicles in a manner similar to that observed for fatty acids [1–4] resulting in equilibration of surface charge between the vesicle populations and the disassociation of aggregates formed by ionic attractions between the positively and negatively charged vesicles of the initial mixture. Here we show this to be the case. Further, we have previously demonstrated that the transbilayer distribution of stearylamine and fatty acids is sensitive to the transmembrane pH gradient [10]. The concentration

Abbreviations: OA, oleic acid; SA, stearylamine; DPPC, dipalmitoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)dioleoylphosphatidylethanolamine; Rh-PE, *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine; LUV, large unilamellar vesicle; FATMLV, freeze and thawed multilamellar vesicle.

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of these lipids in the outer monolayer of vesicles can be reduced by several orders of magnitude depending upon the ΔpH across the membrane. Here, we describe the influence of pH gradients on the association-disassociation process described above as well as the intervesicle flux of fatty acid and stearylamine modulated by manipulating proton gradients across donor and/or acceptor vesicles.

Materials and Methods

Lipids

Dioleoylphosphatidylcholine (DOPC) and *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine (Rh-PE), *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)dioleoylphosphatidylethanolamine (NBD-PE), and brain phosphatidylserine (PS) were obtained from Avanti Polar Lipids (Birmingham, AL).

Stearylamine (SA) and oleic acid (OA) were purchased from Sigma (St. Louis) while the [^{3}H]dipalmitoylphosphatidylcholine (DPPC) was purchased from NEN. [^{14}C]Oleic acid was obtained from ICN and the [^{14}C]stearylamine was obtained from Dr. J. Wilschut. Lipid compositions of vesicles are expressed as molar ratios.

Vesicles

Large unilamellar vesicles were prepared by extrusion through polycarbonate filters [11] using the Lipex Extruder obtained from Lipex Biomembranes Inc., Vancouver, Canada. Lipid mixtures were dried down from chloroform under a stream of nitrogen gas and residual chloroform was removed under vacuum for one hour. The appropriate buffer composed of either 150 mM sodium citrate, 10 mM Hepes, 5 mM K_2SO_4 (pH 7.4) or 150 mM sodium citrate, 5 mM K_2SO_4 (pH 4.0) was added to the lipid film and vortexed to prepare a liposomal suspension of approx. 10 μmol/ml total phospholipid. The liposomes were freeze-thawed five times using liquid nitrogen-warm water cycles in order to increase the trapped volume of the vesicles and to promote equilibrium solute distributions. These freeze and thawed multilamellar vesicles (FATMLVs) [12] were then extruded ten times through two stacked 0.1 μm polycarbonate filters (Nuclepore). After preparation, all vesicles were passed down Sephadex G-50 columns equilibrated with 10 mM Na_2SO_4, 10 mM Hepes, 5 mM K_2SO_4 (pH 7.4). Exchange of the initial hydrating buffer for the low ionic strength buffer was necessary to enable binding of charged vesicles to the ion exchange columns.

Turbidity experiments to monitor vesicle aggregation

Vesicles (DOPC/PS (8 : 2) or DOPC/SA (9.5 : 0.5)) were prepared as described above by hydrating the lipid films in buffer containing 150 mM citrate (pH 7.4). After exchanging the external buffer employing G-50 columns the turbidity of both sets of vesicles (at a concentration of 1 μmol/ml total phospholipid) was monitored at 550 nm using a Shimadzu UV-160 spectrophotometer.

Vesicles containing PS were then added into a cuvette to a final concentration of 0.5 μmol/ml. After monitoring the turbidity of the solution for approx. 10 s, stearylamine-containing vesicles (0.5 μmol/ml final concentration) were added and mixed. The change in absorbance was then measured over the next 2 min.

Effects of a transmembrane pH gradient on turbidity

Vesicles composed of DOPC/PS (8 : 2) or DOPC/SA (9.5 : 0.5) were prepared as described above except vesicles containing SA were hydrated in a buffer containing 150 mM citrate (pH 4.0). The turbidity of both sets of vesicles were measured separately. PS-containing vesicles were added to the cuvette to a final concentration of 0.5 μmol/ml. After approx. 20 s stearylamine-containing vesicles were added and mixed. Approx. 40 s later valinomycin and nigericin (0.1 and 0.01 μmol, respectively) were added to the cuvette with mixing.

Stearylamine exchange monitored by ion exchange chromatography using DEAE-Sephacel

Vesicles composed of DOPC/PS/NBD-PE (8 : 2 : 0.1) containing trace quantities of [^{3}H]DPPC or DOPC/[^{14}C]SA/Rh-PE (9.5 : 0.5 : 0.1) were prepared in 150 mM citrate, 10 mM Hepes, 5 mM K_2SO_4 buffer (pH 7.4) and passed down Sephadex G-50 columns equilibrated with 10 mM Na_2SO_4, 10 mM Hepes, 5 mM K_2SO_4 buffer (pH 7.4). 1 μmol of each set of vesicles were passed down DEAE-Sephacel (Pharmacia) columns equilibrated in external buffer (10 mM Na_2SO_4, 10 mM Hepes, 5 mM K_2SO_4 (pH 7.4)). After two fractions (approx. 3.5 ml per fraction) were collected, the eluting buffer was changed (500 mM NaCl, 10 mM Hepes (pH 7.4)) and four more fractions were collected. After establishing elution profiles for the two vesicle populations, 1 μmol of each population of vesicles were added together, mixed, and allowed to incubate for 5 min at room temperature (approx. 20 °C). The mixture of vesicles was then applied to a DEAE-Sephacel column and eluted under the same conditions as described above. Radioactive decays were monitored using a Packard Tricarb 2000 CA liquid scintillation counter. The presence of fluorescent lipid was assayed employing an SLM Aminco SPF 500C fluorometer.

Effects of ΔpH on stearylamine exchange

Brain phosphatidylserine-containing vesicles ([^{3}H]-DOPC/PS or DOPC/PS (8 : 2)) and stearylamine-containing vesicles (DOPC/[^{14}C]SA or [^{3}H]DOPC/[^{14}C]-SA (9.5 : 0.5)) were prepared as described above. The PS-containing vesicles were hydrated at pH 7.4 while

the SA-containing vesicles were hydrated at pH 4.0. Vesicles (1 μmol of each population) were mixed ([^{3}H]-DOPC/PS + DOPC/[^{14}C]SA or DOPC/PS + [^{3}H]-DOPC/[^{14}C]SA) in the pH 7.4 buffer and incubated either in the presence or absence of the ionophores valinomycin and nigericin (0.1 and 0.01 μmol, respectively). After a 5 min of incubation at room temperature, the vesicle populations were separated on DEAE columns and the fractions were assayed for ^{3}H and ^{14}C.

Oleic acid exchange

Oleic acid containing vesicles (DOPC/PS/[^{14}C]OA (8:2:0.5)), DOPC vesicles and DOPC vesicles containing stearylamine (DOPC/SA (9.5:0.5)) were hydrated at pH 10.0 (100 mM Na_2SO_4, 50 mM H_3BO_3, 5 mM K_2SO_4) or pH 7.0 (150 mM sodium citrate, 10 mM Hepes, 5 mM K_2SO_4) as required. After extrusion, vesicle populations were passed down G-50 columns equilibrated in pH 7.0 external buffer (20 mM Na_2SO_4, 10 mM Hepes, 5 mM K_2SO_4). 1 μmol of each vesicle population were mixed either with or without the presence of ionophores, and after a 5 min incubation at room temperature the populations were separated on DEAE-Sephacel columns as described above. Fractions from the column were then counted employing a dual label program on a Packard 2000 CA liquid scintillation counter.

Results

The aggregation-disaggregation behaviour of vesicles consisting of DOPC and 20 mol% PS and donor vesicles composed of DOPC and 5 mol% stearylamine is shown in Fig. 1. The turbidity profiles of vesicles prepared with a transmembrane $\Delta pH = 0$ (pH 7.4 inside and outside) demonstrate that separately PS and stearylamine-containing vesicles are only slightly turbid, giving an absorbance of approx. 0.06 at 550 nm. However,

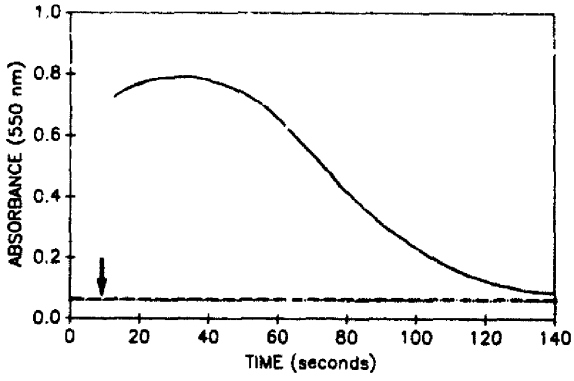

Fig. 1. Turbidity measurements at 550 nm of (a) (- - -) (DOPC/PS (8:2) vesicles (1 μmol/ml), (b) (-·-·-·-) DOPC/stearylamine (9.5:0.5) vesicles (1 μmol/ml) and (c) (———) a mixture of 0.5 μmol/ml of the two vesicle populations. The arrow indicates the time at which the SA-containing vesicles were added (see Methods).

when mixed the turbidity of the solution immediately increases then slowly decreases until it reaches the original turbidity of the separate vesicles. This process can also be followed visually. It is logical to suggest that this aggregation-disaggregation phenomenon occurs due to an initially electrostatic attraction giving rise to aggregation of the oppositely charged vesicles during which time exchange of the stearylamine occurs [13], followed by disaggregation of the vesicles after the surface charges of acceptor and donor vesicles have equilibrated. This interpretation is supported by the data shown in Fig. 2. Figs. 2(a) and 2(b) show the elution profiles from DEAE-Sephacel columns of [^{3}H]DOPC/PS (8:2) and DOPC/[^{14}C]SA (9.5:0.5) vesicles, respectively. In Fig. 2(c) the two vesicle populations were mixed and incubated for 5 min before being applied to the DEAE column. All the tritium counts are associated with the PS-containing vesicles, indicating that there has been no exchange of [^{3}H]DPPC. The [^{14}C]stearylamine counts on the other hand are approximately equally distributed between the two vesicle populations. These results clearly suggest that only stearylamine is undergoing exchange in this vesicle system. It was also of interest to incorporate the fluorescent energy transfer probes rhodamine-PE and NBD-PE into the stearylamine and PS-containing vesicles, respectively, in order to ascertain whether there was evidence of lipid mixing and fusion during aggregation. The results, shown in Fig. 2(d), show that these probes do not exchange between the acceptor and donor vesicles during the aggregation stage. Moreover, there was no detection of fluorescence energy transfer during the aggregation-disaggregation reaction.

Having established that stearylamine rapidly equilibrates between the two vesicle populations in the absence of a transmembrane pH gradient, vesicles of DOPC/SA (9.5:0.5) were prepared with an internal pH of 4.0. Given the external pH of 7.4, a pH gradient of −3.4 units (calculated as inside pH − outside pH) is then formed across the bilayer. We have shown that such a gradient induces stearylamine to accumulate at the inner monolayer of the vesicle by reducing the outer monolayer concentration of stearylamine by an estimated three orders of magnitude [10]. Fig. 3 demonstrates the change in the outer surface charge associated with the stearylamine-containing vesicles in the presence of a pH gradient (acidic inside). When DOPC/PS (8:2) and DOPC/stearylamine (9.5:0.5) vesicles are mixed there is no aggregation, consistent with an absence of a positive surface charge on the DOPC/stearylamine vesicles. However, dissipating the gradient using a combination of nigericin, a proton ionophore, and valinomycin, a K^+ onophore, in the presence of K^+ ions allows stearylamine to equilibrate across the bilayer. A positive surface charge is then restored and aggregation with the negatively charged PS-containing vesicles is observed as an increase in

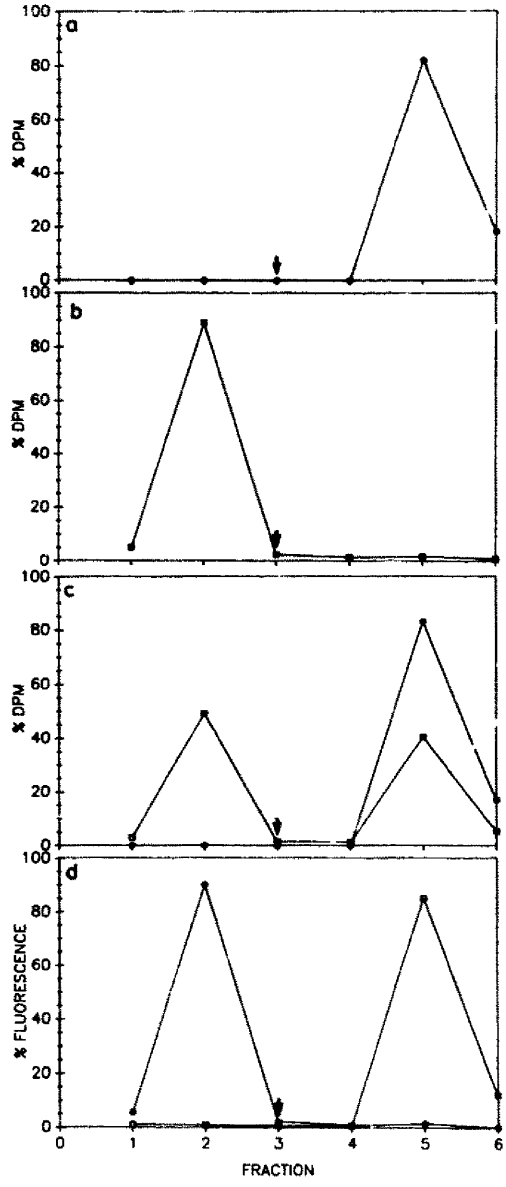

Fig. 2. Elution profiles from DEAE-Sephacel columns of (a) [^{3}H]DOPC/PS (8:2) vesicles, (b) DOPC/[^{14}C]stearylamine (9.5:0.5) vesicles, (c) a mixture of vesicles in (a) and (b), and (d) a mixture of DOPC/PS/NBD-PE (8:2:0.1) and DOPC/stearylamine/Rh-PE (9.5:0.5:0.1) vesicles. ●, ^{3}H counts, □, ^{14}C counts. In panel d, (●) indicates rhodamine fluorescence (ex. 560, em. 590) and (□) indicates NBD fluorescence (ex. 480, em. 530). The arrow indicates the point at which elution with high ionic strength buffer was started.

turbidity. Subsequently, inter-vesicle exchange of stearylamine takes place, and the vesicles disaggregate. This mechanism is confirmed in Fig. 4 which demonstrates that in the presence of a pH gradient (ΔpH = −3.4) when stearylamine is located at the inner monolayer of the vesicle, exchange of stearylamine between vesicles does not occur. Specifically, [^{3}H]DOPC/PS LUVs were incubated with DOPC/[^{14}C]stearylamine LUVs (pH 4.0 inside) and after 5 min incubation,

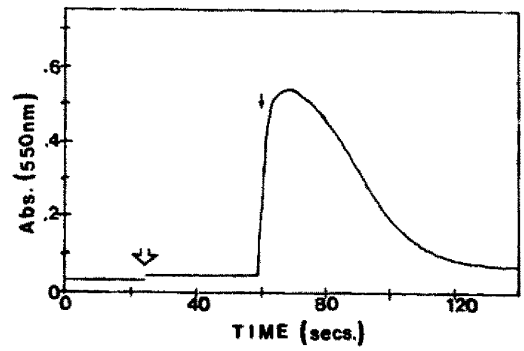

Fig. 3. Turbidity measurements at 550 nm of a mixture of DOPC/PS (8:2) and DOPC/stearylamine (9.5:0.5) vesicles with a transmembrane pH gradient (ΔpH = −3.4). DOPC/PS vesicles added at t = 0; ⇓, addition of DOPC/stearylamine vesicles; ↓, addition of ionophores (nigericin and valinomycin) to collapse the pH gradient.

separation on an ion exchange column shows that 97% of the stearylamine is still associated with the DOPC vesicles. However, as illustrated in Fig. 4(b), in the presence of ionophores stearylamine equilibrates with the PS-containing vesicles. It should be noted that exchange of phospholipid label between the vesicle systems was never observed.

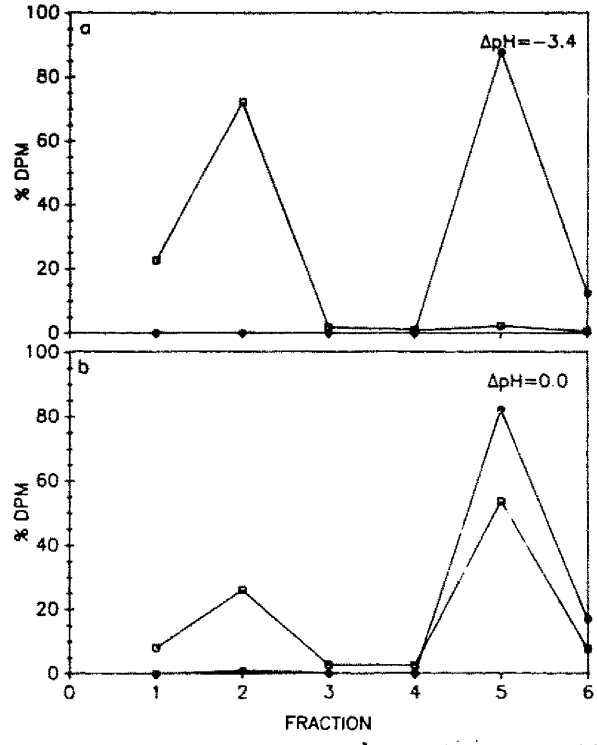

Fig. 4. Elution profiles of mixtures of [^{3}H]DOPC/PS (8:2) vesicles and DOPC/[^{14}C]stearylamine vesicles. (a) DOPC/[^{14}C]stearylamine vesicles have a transmembrane pH gradient (ΔpH = −3.4) acidic interior. (b) The same vesicle mixture plus the ionophores, nigericin and valinomycin, used to collapse the transmembrane pH gradient. ●, ^{3}H counts; □, ^{14}C counts.

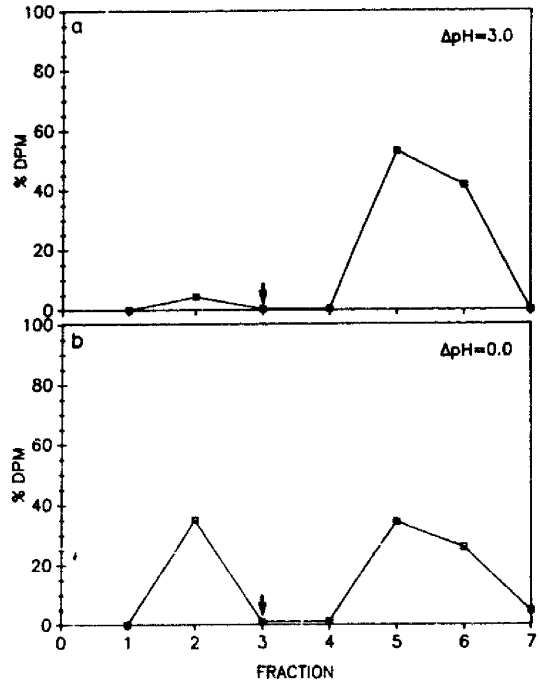

Fig. 5. Elution profiles from DEAE-Sephacel columns of mixtures of DOPC/SA and DOPC/PS/[^{14}C]OA (8:2:0.5) vesicles. (a) The oleic acid-containing vesicles has a basic transmembrane pH gradient (ΔpH = 3.0). (b) The vesicles in the presence of ionophores to collapse the pH gradient. □, ^{14}C counts. The arrow indicates the point at which elution with high ionic strength buffer was started.

The results presented above deal with the transfer of stearylamine between vesicles. Vesicles exhibiting a positive ΔpH (basic inside) sequester fatty acids to the inner monolayer [10], and consequently fatty acid exchange between membranes should also be influenced by transmembrane proton gradients. In order to monitor the exchange of fatty acid between two vesicle populations and test this hypothesis we studied the following systems. In the first DOPC/SA (9.5:0.5) vesicles were incubated with DOPC/PS/[^{14}C]OA (8:2:0.5) LUVs. Vesicles containing oleic acid (OA) were prepared with a pH gradient that was basic inside (see Methods) yielding a ΔpH = 3.0. As we have described above (see Figs. 1 and 3), mixing positively charged stearylamine-containing vesicles and negatively charged PS-containing vesicles results in immediate aggregation followed by a slower disaggregation due to the equilibration of stearylamine between the vesicle populations. The same phenomenon was observed for DOPC/SA and DOPC/PS/[^{14}C]OA vesicle systems. However, as shown in Fig. 5(a), the exchange of oleic acid is greatly reduced by the presence of a positive ΔpH. It is worthwhile pointing out that despite vesicle aggregation and stearylamine exchange the bulk of the oleic acid remains associated with the inner monolayer of the DOPC/PS/OA vesicles. This suggests that vesicle aggregation and lipid exchange does not significantly enhance the proton permeability of the PS-containing vesicles, otherwise the ΔpH would collapse enabling oleic acid to exchange. When the ΔpH is deliberately dissipated employing the ionophores valinomycin and nigericin in the presence of K^+, [^{14}C]oleic acid is observed to elute from the DEAE column in both vesicle fractions indicating equilibration of the fatty acid between DOPC/SA and DOPC/PS/OA vesicles (Fig. 5(b)).

The second approach involved monitoring the transfer of fatty acid from one population of vesicles to another in non-aggregating systems as shown in Fig. 6. LUVs composed of [^{3}H]DOPC were incubated with vesicles of DOPC/PS/[^{14}C]OA (8:2:0.5). The results of Fig. 6(a) show that in the absence of a ΔpH oleic acid rapidly equilibrates between the two vesicle populations. However, when the DOPC LUVs exhibit a ΔpH of 3.0 units (inside basic) oleic acid preferentially moves into the DOPC vesicles (Fig. 6(b)). At equimolar concentrations of donor and acceptor vesicles, 85% of the oleic acid transfers to the DOPC vesicles. At higher ratios of acceptor to donor vesicles more than 90% of the oleic acid transfers to the DOPC vesicles.

The above experiments clearly demonstrate an ability of transmembrane pH gradients to modulate lipid flow between membranes. However, a large proportion of free fatty acid in vivo is delivered to peripheral tissues

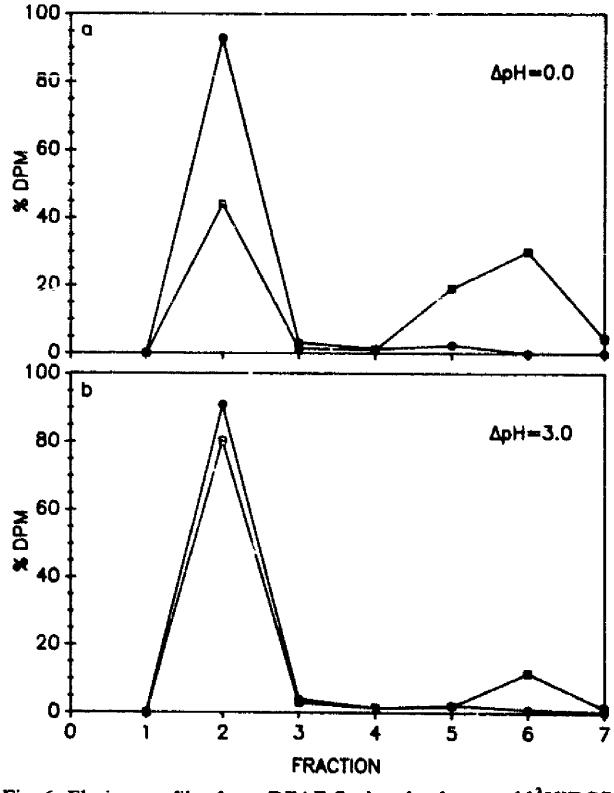

Fig. 6. Elution profiles from DEAE-Sephacel columns of [^{3}H]DOPC, DOPC/PS/[^{14}C]OA vesicle mixtures. (a) Neither vesicle population has a transmembrane pH gradient. (b) The [^{3}H]DOPC vesicles have a basic transmembrane pH gradient (ΔpH = 3.0). ●, ^{3}H counts; □, ^{14}C counts.

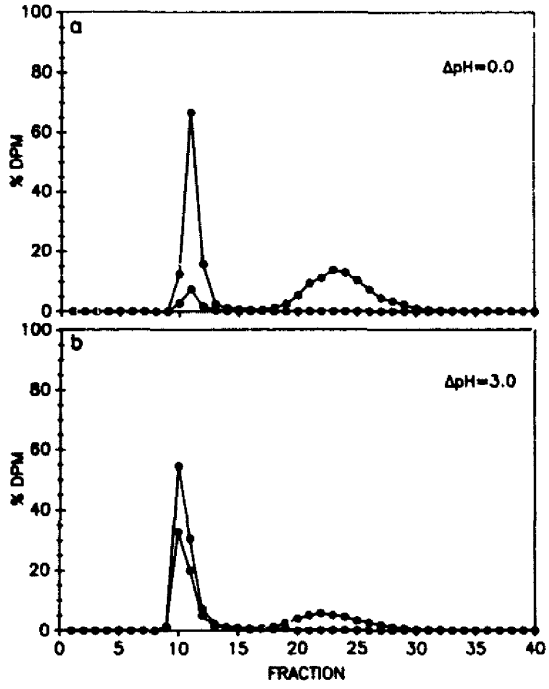

Fig. 7. Extraction of [^{14}C]OA from fatty acid-loaded bovine serum albumin (BSA) by DOPC vesicles. Separation of vesicles and BSA on Sepharose CL-4B columns. (a) [^{3}H]DOPC vesicles without a transmembrane pH gradient (ΔpH = 0.0). (b) [^{3}H]DOPC vesicles with a basic transmembrane pH gradient (ΔpH = 3.0). ○, ^{3}H counts; ●, ^{14}C counts. BSA elutes between fractions 18–28.

bound to serum albumin. In the light of our observations on the modulation of fatty acid flow between membranes by transmembrane pH gradients, it was of interest to see whether albumin could be depleted of fatty acid when incubated with a population of vesicles that exhibit a positive ΔpH (inside basic). Fig. 7 presents the data from such an experiment. When incubated with vesicles composed of DOPC (ΔpH = 0) fatty acid equilibrates between protein and membrane as shown in Fig. 7(a). However, in the presence of a positive ΔpH, net flux of fatty acid occurs in the direction of the vesicles, significantly depleting albumin of lipid (see Fig. 7(b)).

Discussion

The results presented here illustrate the remarkable exchange abilities of stearylamine and oleic acid and the sensitivity of these exchange characteristics to transmembrane pH gradients across vesicle membranes. Here we discuss the mechanisms modulating these exchange processes and their implications for lipid exchange in vivo.

The aggregation and subsequent disassociation processes observed for positively charged (DOPC/SA) and negatively charged (DOPC/PS) vesicles provides a graphic illustration of rapid intervesicular exchange of stearylamine. There are three points of interest.

First, with regard to the exchange mechanism, whereas the initial attraction between positively and negatively charged vesicles is sufficiently intense to produce visible flocculation under our experimental conditions, neither membrane fusion nor exchange of diacylphospholipids was observed. The lack of phospholipid exchange indicates that intervesicular mixing between external monolayers, commonly observed in fusing systems [14] does not occur and that the bilayers of these aggregated vesicles remain intact. This is also supported by the observation that the proton permeability barrier for acceptor vesicles is maintained during the aggregation-disaggregation process involving exchange of stearylamine (Fig. 5). Thus exchange of stearylamine may be most logically suggested to proceed via intermediary partition of SA into the aqueous phase separating the vesicles. In the case of oleic acid, it is widely accepted that non-mediated lipid exchange occurs via this mechanism, involving desorption of monomers from the bilayer into the aqueous solution, and subsequent diffusion to an acceptor site [1–4]. This is also supported by the result presented here for non-aggregating systems (Fig. 6) where oleic acid is observed to rapidly equilibrate between the two vesicle populations.

The second point concerns the non-exchangeable characteristics of the fluorescent lipids rhodamine-PE and NBD-PE. As indicated in Fig. 2, even under conditions of vesicle aggregation and stearylamine exchange, there is no evidence of lipid mixing as reported by these probe molecules. This provides an additional confirmation of indications that these fluorescent lipids do not readily exchange between aggregated systems [14,15], supporting their use as non-exchangeable markers in studies of membrane fusion.

The third point concerns the modulation of stearylamine and oleic acid diffusion by transmembrane pH gradients. These phenomena are relatively straightforward to understand in the light of previous observations [10] that lipophilic weak bases, such as stearylamine, will partition to the inner monolayer in vesicles exhibiting a transmembrane pH gradient (inside acidic). Alternatively, lipophilic weak acids such as oleic acid will partition to the inner monolayer when the interior is basic [10]. Thus the absence of aggregation and lipid exchange when appropriate pH gradients are employed is a graphic consequence of the ΔpH-dependent lipid asymmetry.

Modulation of lipid exchange by transmembrane pH gradients may also occur in vivo. For example, the results presented here show that fatty acid can be induced to transfer from one population of vesicles to another by simply maintaining a basic interior pH in the acceptor vesicles. Such vesicles can also induce fatty acid depletion of bovine serum albumin. In vivo, most fatty acid movement across membranes occurs via passive diffusion. Indeed, serum and cytoplasmic pH val-

ues are strictly maintained within narrow limits [17]. However, in the case of organelles such as the lysosome, which maintain an acidic interior [7,10], fatty acid would be expected to mainly reside in the outer (cytoplasmic) monolayer. Phospholipid asymmetry, common to many plasma membranes [18] may also play a role. Phosphatidylserine, for example, is negatively charged and frequently located in the cytoplasmic monolayer of cell membranes, resulting in a negative surface potential [19]. This potential will repel anions from and attract cations to the lipid/water interface and will result in a significantly lower pH at the cytoplasmic membrane surface when compared to the exterior bulk pH. Such a gradient could be important in enhancing the flow of fatty acids out of adipocytes, for example.

We and others have previously discussed the possibility of fatty acid flow from lysosomes into the cytoplasm [7,10] because of the acidic pH gradient across the lysosomal membrane. Mitochondria, on the other hand, develop a basic internal pH during oxidative phosphorylation [20]. Given the results presented here this might be expected to result in an accumulation of free fatty acids by mitochondria. However, cells have evolved a complex system by which fatty acids are first activated and subsequently converted to carnitine derivatives which are then transported into the mitochondrial matrix. The data described here suggest that intracellular free fatty acid may be deliberately kept at a very low concentration to prevent these lipids accumulating within organelles, such as the mitochondria, in an unregulated manner. The potential harm of this type of accumulation is illustrated by the ability of low concentrations of lysosomotropic detergents to kill cells [21]. These molecules are aliphatic amines which accumulate in lysosomes in response to the pH gradient, disrupt membrane integrity and cause the release of lysosomal enzymes into the cytoplasm.

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