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Calcitonin-induced changes in the organization of sulfatide-containing membranes

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The interactions of salmon calcitonin with glycosphingolipid sulfatide are studied by right angle light scattering from the lipid suspension, by the excimer to monomer ratio (E/M) of the fluorescence intensity of pyrene phosphatidylcholine and pyrene sulfatide and by the leakage of carboxyfluorescein. It was found that calcitonin strongly modified the structure of the sulfatide aggregate, as indicated by the light scattering determinations. At a lipid peptide ratio 100:1 (molar ratio) light scattering from the suspension was negligible, indicating the formation of peptide-sulfatide complexes with a structure different from that of the lipid aggregate. The interactions of calcitonin with sulfatide when the latter is a component of a bilayer were also evaluated. A specific calcitonin–membrane sulfatide interaction was demonstrated by determining the temperature-dependent E/M of pyrene phosphatidylcholine and pyrene sulfatide in dipalmitoyl phosphatidylcholine/sulfatide (80:20, molar ratio) liposomes. The E/M curves were modified by calcitonin only when the liposomes were labelled with fluorescent sulfatide which probes the sulfatide behavior in the membrane. Furthermore, the addition of calcitonin to the incubation medium of liposomes containing sulfatide promoted the release of vesicle entrapped carboxyfluorescein without disrupting the bilayer structure, the release being correlated with the amount of sulfatide in the bilayer and the calcitonin concentration in the medium.

Introduction

The interactions of proteins with phospho- and glycolipids are of particular interest because they have been considered important for many membrane functions, or membrane-related phenomena such as the fusion and binding of ligands to the cell surface. In previous papers we demonstrated that the acidic glycosphingolipid sulfatide can strongly interact with albumin [1] and insulin [2] at acidic pH values, a condition under which these proteins are positively charged. Albumin, in particular, promotes the aggregation and fusion of sulfatide-containing vesicles through glycolipid–protein interactions which depend on the sulfatide concentration in the bilayer. In the case of insulin, it is the binding to the glycolipid at acidic pH (3.5), as consequence of ionic interactions, which determines a stable association of the protein with liposomes containing sulfatide. This association remains even when electrostatic interactions are removed by the neutralization of the incubation media, which transforms insulin into its anionic form.

Many peptide hormones, including glucagon [3], β-endorphin [4], growth hormone [5] and calcitonin [6], interact with lipids through conformational changes, resulting in an amphipatic structure of the peptide. In fact, a specific affinity for acidic lipids has been demonstrated for peptide hormones which are positively charged at neutral pH, i.e., β-endorphin [4] and calcitonin [6]. This affinity is due to electrostatic interactions which promote the formation of lipoprotein complexes in which peptides assume an amphipatic structure. Amongst the lipids reported in literature, sulfatide has been considered also; it has been hypothesized that interactions of sulfatide with β-endorphin could be relevant to the biological activity of the peptide [4,7]. In this study we proposed to characterize the calcitonin to sulfatide binding, already reported by Epand et al. [8]. Particular attention was paid to the interaction of peptide with sulfatide when the latter is a component of a phosphatidylcholine bilayer; possible modifications of membrane properties as a consequence of these interactions were also investigated. The affinity of calcitonin for membrane sulfatide could be involved in elucidating the mechanism of peptide activity at the level of the central nervous system which is particularly rich in acidic glycolipids such as gangliosides and sulfatides [9,10]; in particular, it has been demonstrated that sulfatide, which is mainly located in myelin, is also present in isolated neurons [11] and in

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synaptosomal plasmamembranes [11,12]. Since sulfatide is naturally located almost exclusively in the outer layer of the membrane [13], unlike acidic phospholipids which are mainly present in the inner layer [14], sulfatide could be involved in the calcitonin-to-cell membrane binding processes.

Materials and Methods

Analytical grade chemicals, distilled solvents and doubly distilled water were used. Dipalmitoylphosphatidylcholine (DPPC), egg phosphatidylcholine (PC), 5(6)-carboxyfluorescein (CF), NADH and synthetic salmon calcitonin (CTs) were purchased from Sigma; 1-palmitoyl-2-(10-pyrene)decanoyl phosphatidylcholine ($P_{10}PC$) and pyrene decanoic acid were obtained from Molecular Probe (Junction City, OR); Sepharose 4B from Pharmacia (Uppsala Sweden). Bovine brain sulfatide (CS) was purified following the procedure of Hara and Radin [15]. *N*-(12-(1-Pyrene)decanoyl) galactosyl-sphingosine I^3-sulfate ($P_{10}CS$) and *N*[^{14}C]stearoylgalactosyl-sphingosine I^3-sulfate were prepared from galactosyl-sphingosine I^3-sulfate following the procedure of Marchesini et al. [16]

Preparation of sulfatide and sulfatide-calcitonin aqueous dispersions

Sulfatide dissolved in chloroform/methanol 2:1 was evaporated under a nitrogen stream, dried under vacuum for 30 min and resuspended at 70°C in 1 ml of 10 mM Tris-HCl buffer (pH 7.4) containing the appropriate amount of NaCl or urea. Sulfatide-calcitonin codispersions were obtained by adding the appropriate amount of calcitonin to the sulfatide suspending medium, following the procedure described above for sulfatide resuspension. The final concentration of sulfatide was always 10^{-4} M. The obtained suspensions were used for light scattering determinations 20 min after preparation.

Preparation of phosphatidylcholine / sulfatide small unilamellar vesicles

Different lipid mixtures dissolved in chloroform/methanol (2:1, v/v) were evaporated under a nitrogen stream, dried under vacuum for 30 min and resuspended above the lipid transition temperature (T_m) in the appropriate buffer to obtain multilamellar vesicles that were then subjected to sonication to obtain small unilamellar vesicles, following the procedure of Barenholz et al. [17] and already applied by us to sulfatide containing vesicles [18].

Excimer-to-monomer ratio determinations

Phase transition curves of DPPC-CS (80:20, molar ratio) small unilamellar vesicles were determined by the Excimer (475 nm)-to-monomer (379 nm) fluorescence intensity ratio (E/M) of $P_{10}PC$ or $P_{10}CS$ at different temperatures [16]. The final lipid concentration was 10^{-4} M and in both cases the probe represented 3.5 mol% of the total lipids. Lipid mixtures were resuspended above the T_m in 10 mM Tris-HCl, 154 mM NaCl (pH 7.4) or in the same buffer containing 2 mM of calcitonin ($2 \cdot 10^{-5}$ M).

Carboxyfluorescein leakage determinations

Small unilamellar vesicles of egg phosphatidylcholine/sulfatide at different molar ratios were prepared in 10 mM Tris-HCl (pH 7.4), 100 mM CF. Vesicles containing entrapped CF were separated from extravesicular CF by passing the mixture through a 1.5 × 20 cm Sepharose 4B column at room temperature; 100 μl of eluted vesicles, corresponding to 100 nmol of lipids, were then diluted to 1 ml with 10 mM Tris-HCl, 154 mM NaCl (pH 7.4) and, setting the excitation wavelength at 490 nm, the fluorescence emission at 520 nm was determined (zero time fluorescence, F_0).

The increase in the fluorescence emission of CF, a highly fluorescent substance which undergoes a concentration-dependent self-quenching [20], was monitored by measuring the time-dependent leakage of this molecule from liposomes. The data are expressed as % CF release: $100 \times (F - F_0)/(F_t - F_0)$, where F is the fluorescence intensity at a given time and F_t is the total CF fluorescence measured after the rupture of vesicles with sodium dodecyl sulfate (SDS), 2% final concentration.

Light scattering measurements

Light scattering experiments were carried out with a Jasco FP 700 spectrophotofluorimeter. The Rayleigh peak from unpolarized incident light at 450 nm was measured to monitor the 90° light scattering of the lipid suspension. The data are expressed as A/A_0 where A_0 is the light scattering of the reference sample and A that of the analyzed sample.

Fluorescence measurements

All fluorescence measurements were carried out with a Jasco FP 700 spectrophotofluorimeter equipped with a cuvette holder the temperature being mantained by a Haake GD3 thermostatic circulating bath and monitored with a Subline PT 100 digital thermometer.

Gel chromatography separations

Egg phosphatidylcholine/sulfatide (80:20, molar ratio) small unilamellar vesicles containing entrapped carboxyfluorescein were incubated for 20 min, either alone or in the presence of calcitonin, and then subjected to gel filtration on Sepharose 4B (1.5 × 20 cm) using 10 mM Tris-HCl, 154 mM NaCl as elution buffer; 1 ml fractions were collected and each fraction ana-

lyzed for CF, egg phosphatidylcholine and sulfatide content. The elution profile of CF was determined by measuring the total fluorescence intensity after rupturing the vesicles with SDS 2% final concentration. The phospholipid elution profile was determined by measuring organic phosphorus according to Bartlett [21]. To determine the elution profile of sulfatide, trace amounts of [^{14}C]stearoylsulfatide were added to the chloroform/methanol lipid solution in order to obtain a sulfatide specific radioactivity of 500 000 dpm/μmol; thus, the sulfatide content in each fraction was determined radiometrically.

Results and Discussion

The ability of salmon calcitonin to interact with sulfatide is demonstrated by the right angle light scattering determinations reported in Fig. 1. The codispersion of sulfatide with increasing amounts of calcitonin determined a progressive reduction of the light scattering from the suspension, expressed as A/A_0 where A is the value obtained at a given calcitonin/sulfatide molar ratio and A_0 is the value obtained for the pure sulfatide dispersion. A 50% decrease in the light scattering was obtained when the calcitonin concentration in the medium was 0.75 μM and the light scattering

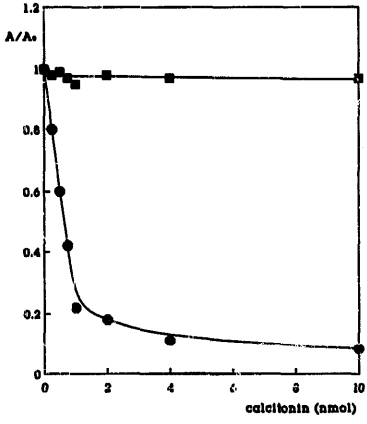

Fig. 1. Decrease of right angle light scattering from sulfatide (●) and cerebroside (■) suspensions as a function of increasing amounts of calcitonin in 1 ml of the resuspending medium (final lipid concentration 10^{-4} M). Light scattering determinations are expressed as A/A_0, where A is the value obtained at a given calcitonin concentration and A_0 is the value obtained from a pure lipid dispersion.

was pratically negligible above 1 μM calcitonin. The lipid-peptide complex formed is quite a stable structure since light scattering values from the suspensions did not change after 24 h or even longer. When calcitonin was added to the resuspending medium of cerebroside, a neutral glycolipid which differs from sulfatide for the absence of the sulfate group, no changes in the light scattering from the suspension were observed even at high concentrations of calcitonin (Fig. 1).

Light scattering determinations reported in Fig. 1 clearly indicate that calcitonin has a strong affinity for the acidic glycolipid sulfatide, which results in a dramatic change in the morphology of sulfatide aggregates. These results are in good agreement with those obtained by Epand et al. [8] who reported, on the basis of electron microscopy results, the possibility that calcitonin could interact with sulfatide at a lipid/peptide ratio of 10:1 without any increase in the helical content of the peptide since the 222 nm ellipticity of the CD spectrum on calcitonin did not increase.

Light scattering determinations reported here demonstrate that the morphology of sulfatide aggregates is strongly modified in the presence of very low concentrations of calcitonin. An almost complete lipid solubilization was obtained with a lipid/peptide ratio of 100:1, demonstrating that only a few molecules of calcitonin are required to give lipopeptide complexes with a more hydrophilic structure than sulfatide aggregates. Results obtained with cerebroside indicate that the negative charge is necessary in order to determine the lipid–protein interactions. These interactions could promote conformational changes in the peptide and bring about a segregation of hydrophobic amino acids in domains which can in turn, interact with the hydrophobic moiety of the glycolipid.

The importance of electrostatic interactions in the formation of lipid-peptide complexes is further demonstrated by results reported in Fig. 2: increasing the NaCl concentration from 0 to 1 M greatly affected on the light scattering from the lipid-peptide complex. Since the light scattering from the pure sulfatide suspension increased with increasing the ionic strength of the resuspending medium, the data of the calcitonin-sulfatide complex (1:100, molar ratio) at different NaCl or urea concentrations are related to the light scattering A_0 from the pure sulfatide dispersion in the same resuspending medium. The data reported in Fig. 2 indicate that for NaCl concentrations higher than 154 mM the light scattering from the calcitonin-sulfatide complex rapidly increases and, above 500 mM NaCl, A/A_0 approaches the value of 1, thus indicating that high salt concentrations are able to decrease the strength of electrostatic interactions, reduce the extent of CT-sulfatide complexation. The presence of high concentrations of urea in the medium, which can inhibit hydrogen bonds between the peptide and glycosphingo-

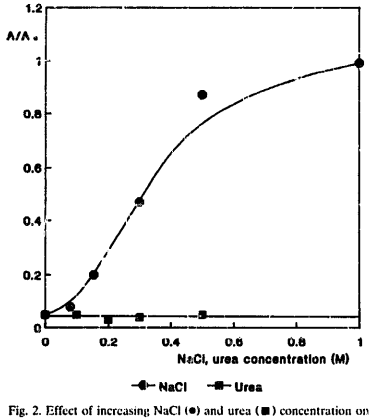

Fig. 2. Effect of increasing NaCl (●) and urea (■) concentration on right angle light scattering values from sulfatide/calcitonin codispersions (100:1, molar ratio). Light scattering determinations are expressed as A/A_0, where A is the value obtained with sulfatide/calcitonin complexes at a given NaCl or urea concentration and A_0 is the value obtained from pure sulfatide dispersion in the same resuspending medium.

lipid, does not interfere with the stability of the peptide-glycolipid complex, thus excluding that this bonding contributes to the lipid–protein interactions necessary to form the complex.

Furthermore, calcitonin is able to disrupt the sulfatide supermolecular organization when added to preformed sulfatide aggregates at different peptide/lipid molar ratios. The time dependent decrease of A/A_0 after calcitonin addition is reported in Fig. 3. The addition of calcitonin to a 10^{-4} M sulfatide suspension determined, in all cases, an initial rapid decrease of the light scattering, followed by a second much slower phase. The addition of 1 nmol of calcitonin promoted, in the first 10 min, a 30% decrease in the light scattering values, expressed as A/A_0. A second addition of 1 nmol of calcitonin determined a further 70% decrease of A/A_0 after 10 min of incubation. When 2 nmol of calcitonin were directly added to a 10^{-4} M sulfatide suspension a 90% decrease in the A/A_0 value was observed after 5 min of incubation. These results confirm that, in the presence of calcitonin, the CT-sulfatide complexes have greater stability than the sulfatide aggregate and that the rate of complex formation depends on the concentration of calcitonin in the medium.

The possibility that calcitonin could interact with sulfatide when the latter is a component of a phospholipid bilayer has also been considered in order to attribute a physiological significance to the great affinity of the polypeptide for this glycolipid. In fact, the natural location of sulfatide in the exofacial layer of cell membrane, together with its tissue distribution, suggests that this molecule has some role in the binding of the hormone to the target cell surface.

When phosphatidylcholine small unilamellar vesicles containing different amounts of sulfatide, from 4 to 20 mol% of total lipids, were incubated in the presence of calcitonin the light scattering properties of the suspension were not modified. This indicates that the bilayer structure of vesicles [6,8] is not disrupted by calcitonin. Furthermore, aggregation or fusion phenomena between sulfatide containing vesicles [1,2] can also be excluded on the basis of these light scattering determinations. It could therefore be hypothesized that the interactions between sulfatide and phosphatidylcholine in the bilayer inhibit the capacity of calcitonin to interact with the acidic glycolipid or, alternatively, that calcitonin still maintains its ability of complexing the sulfatide without macroscopically modifying the bilayer structure of sulfatide-containing small unilamellar vesicles. Evidence strongly supporting the capacity of calcitonin to interact with sulfatide in the bilayer is re-

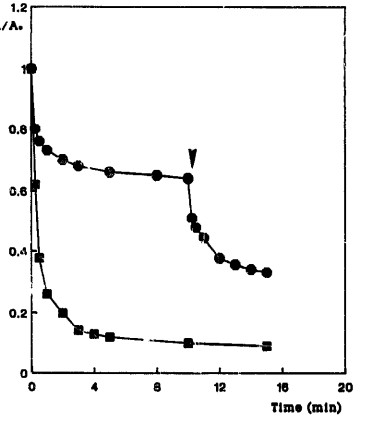

Fig. 3. Effect of calcitonin addition on right angle light scattering from sulfatide aggregates. 1 (●) or 2 (■) nmol of calcitonin were added to sulfatide aggregate (10^{-4} M) and light scattering determined at different times of incubation. The arrow indicate a further addition of 1 nmol of calcitonin to the incubation medium (1 ml). Light scattering values are expressed as A/A_0 where A is the value at a given time and A_0 is that at zero time of incubation.

ported in Fig. 4. The E/M vs. temperature curves of $P_{10}PC$ and $P_{10}CS$ in liposomes of DPPC/sulfatide (80:20, molar ratio) were obtained in the absence and presence of calcitonin. In a previous paper we applied this double labelling of liposomes to discriminate between sulfatide and phospholipid behavior in the membrane [16]; in particular the less pronounced inflection in the E/M curve at the transition temperature obtained with $P_{10}CS$-labelled liposomes was attributed to a partial segregation of $P_{10}CS$ in sulfatide-enriched domains in the membrane, which resulted in a decreased sensitivity of the pyrene ring to the phase transition of the surrounding phospholipids. The results reported in Fig. 4 indicate that when liposomes are labelled with $P_{10}PC$, the E/M curve is not modified by the presence of calcitonin, showing the traditional 'N' shape [22] with a sharp inflection at the transition temperature of DPPC (41.5°C). On the contrary, calcitonin modifies the E/M vs. temperature curves obtained with $P_{10}CS$ labelled liposomes; the E/M curve of $P_{10}CS$ in the presence of calcitonin maintains the inflection at the transition temperature of DPPC, indicating that the phospholipid environment still influences the sulfatide behaviour. This demonstrates that sulfatide is still a component of the liposome; nevertheless, calcitonin determined an increase

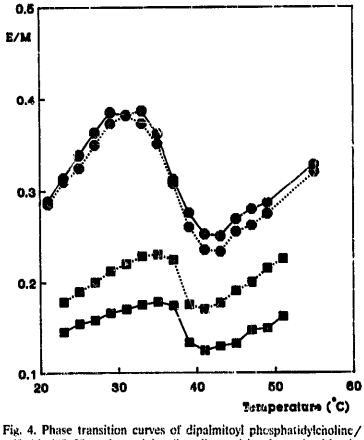

Fig. 4. Phase transition curves of dipalmitoyl phosphatidylcholine/sulfatide (80:20, molar ratio) unilamellar vesicles determined by the excimer (475 nm) to monomer (379 nm) intensity ratio (E/M) of $P_{10}PC$ (●) and $P_{10}CS$ (■) in the absence (——) or in the presence (·····) of $2 \cdot 10^{-6}$ M of calcitonin. In both cases the probe represented 3.5 mol% of total lipids.

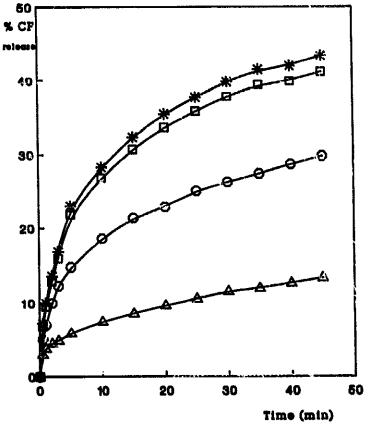

Fig. 5. Extent of calcitonin induced carboxyfluorescein (CF) release from small unilamellar vesicles of egg phosphatidylcholine containing 0 (△), 4 (○), 10 (□) and 20 (•) mol% of sulfatide (total lipid concentration 10^{-4} M). Leakage of CF after addition of 20 nmol of calcitonin ($2 \cdot 10^{-5}$ M), was measured by monitoring the increase of CF fluorescence with time. Data are expressed as % CF release calculated as described in Materials and Methods.

of E/M values both below and above the transition temperature and an increase in the slope of E/M vs. temperature curves in both the gel and liquid-crystalline phases, indicating a greater possibility of sulfatide collisions due to an increase of the glycolipid local concentration in both states of the membrane in the presence of the hormone. The unmodified curves obtained with $P_{10}PC$ confirm the inability of calcitonin to interact with zwitterionic phospholipids.

Though interactions of calcitonin with liposomal sulfatide seem not to disrupt the bilayer structure, it could nevertheless alter the structural lipid organization, thus promoting a modification of membrane properties (i.e., permeability to hydrophilic molecules). To evaluate this possibility we studied the release of liposome entrapped carboxyfluorescein consequent to the addition of calcitonin, both with regard to the sulfatide molar fraction in the liposomal membrane (Fig. 5) and the calcitonin concentration in the incubation medium (Fig. 6).

Fig. 5 shows how calcitonin-dependent membrane permeability to CF is influenced by the presence of different amounts of sulfatide in the bilayer. The kinetics of CF release are characterized by a biphasic behav-

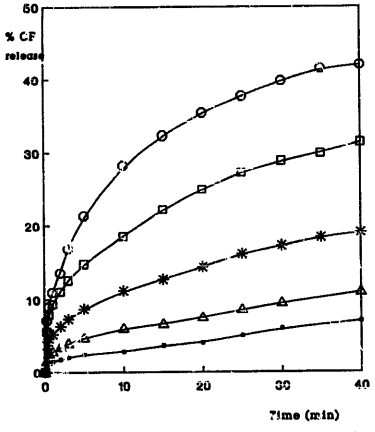

Fig. 6. Extent of carboxyfluorescein release (CF) from egg phosphatidylcholine/sulfatide (80:20, molar ratio) small unilamellar vesicles induced by 2 (■), 4 (▲), 6.5 (•), 15 (□) and 20 (○) nmol of calcitonin. Leakage of CF after addition of calcitonin, was measured by monitoring the increase of CF fluorescence with time. Data are expressed as% CF release calculated as described in Materials and Methods.

ior with an initial fast leakage in the first 4–5 min, followed by a more prolonged slow release which was monitored for up to 45 min.

The spontaneous release of CF from egg phosphatidylcholine small unilamellar vesicles was very low; only 9% of the entrapped molecule was released after 45 min of incubation (data not shown). The presence of different amounts of sulfatide (from 4 to 20 mol%) in the membrane further reduces the spontaneous leakage of the entrapped CF from the liposome; the release after 45 min being 6, 4 and 3% for liposomes containing 4, 10 and 20 mol% of sulfatide, respectively (data not shown). The kinetics of CF spontaneous release still mantain the biphasic behavior obtained in the presence of calcitonin; sulfatide determined a decrease either in the fast and the slow phase of CF release. This could be due to the ordering effect that sulfatide exerts in the polar region of phospholipid bilayers that are in a fluid phase [23,24].

The addition of 20 nmol of calcitonin ($2 \cdot 10^{-5}$ M), while not determining any significant increase in the CF leakage from liposomes of phosphatidylcholine, progressively enhanced the CF release from liposomes containing increasing amounts of sulfatide. With liposomes containing 4 mol% of sulfatide the CF release was 2.5-fold greater than that obtained with egg phosphatidylcholine vesicles in the first 5 min of incubation. The same extent of increase was observed during all incubation periods; after 45 min the CF released from egg phosphatidylcholine liposomes and those containing 4 mol% sulfatide was 12 and 29%, respectively.

A higher mol% of sulfatide in the membrane further increased the calcitonin induced CF release. The leakage from liposomes containing 10 and 20 mol% of sulfatide was, after 5 min, 21.5 and 23%, respectively and, after 45 min, 40.5 and 43%, respectively, thus showing a non linear dependence on the amount of sulfatide in the membrane.

The highest amount of sulfatide used in this experiment was 20 nmol, corresponding to the 20 mol% of total lipids. Since the sulfatide is symmetrically distributed between the inner and the outer layer of the vesicle [23], about 10–12 nmol of the glycolipid are available for calcitonin binding when the peptide is added to the vesicles. Thus, the calcitonin concentration does not represent a limiting factor even in the extreme hypothesis that the optimal ratio for lipid–protein interaction was 1:1. This evidence seems to indicate that a greater number of sulfatide molecules in the bilayer does not determine a proportional increase in the membrane calcitonin binding sites; this is probably due to a different distribution of the sulfatide as a function of its mol% in the membrane. It has been demonstrated, in fact, that sulfatide tends to form glycolipid enriched domains when it is present in a phosphatidylcholine bilayer at concentrations higher than 5 mol% [19,25]. If the sulfatide was randomly dispersed in the membrane at all the mol fractions considered the number of anionic anchoring sites for calcitonin should be proportional to the sulfatide concentration in the membrane, but as a consequence of the segregation of the glycolipid in the membrane, at 10 and 20 mol%, the sulfatide could form polyanionic domains in which more than one negative charge could contribute to the binding site of calcitonin on the membrane, with a consequent deviation from the linearity between sulfatide concentration and CF release.

When liposomes containing 20 mol% of sulfatide were incubated with different amounts of calcitonin, ranging from 2 to 20 nmol, there was a linear increase in the CF released as the calcitonin in the medium was augmented (Fig. 6). The percent release determined at three different times, 15 s, 5 min and 45 min, is always directly proportional to the amount of calcitonin incubated with liposomes containing sulfatide. This seems to indicate that for liposomes containing the same amount of sulfatide, thus the same number of calcitonin binding sites, the modifications in membrane permeability to CF depend on the amount of calcitonin which can interact with the bilayer.

As discussed above, light scattering and E/M determinations demonstrate that the interaction of calcitonin with liposomes containing sulfatide neither disrupts the bilayer structure nor promotes membrane fusion. To exclude the possibility that the interaction of calcitonin with liposomal sulfatide could alter the lipid composition of the vesicles, the liposomes of egg phosphatidylcholine-sulfatide (80:20, molar ratio) with entrapped CF were chromatographed on sepharose 4B after 20 min of incubation with 20 nmol of calcitonin and the elution profile of phosphatidylcholine, sulfatide, and CF was determined. Results are reported in Fig. 7 in which the elution profiles of the same liposome at zero time of incubation are reported for comparison. Phosphatidylcholine and sulfatide were completely recovered in the void volume of the column both at zero and 20 min of incubation and they were present in each fraction at the same mole ratio of the loaded sample. The amount of liposome associated CF was about 95% of the total recovered CF at zero time and decreased to 67% after 20 min of incubation. If the permeability properties of the bilayer were modified without macroscopic alterations of the membrane structure, then it could be possible that the alterations in membrane permeabilty could depend on calcitonin-induced pore formation through the bilayer. If this is true, then hydrophilic entrapped solutes with molecular dimensions greater than the diameter of the pore would be retained by liposomes even in the presence of calcitonin. Preliminary results obtained by substituting CF (PM 358) with NADH (PM 709) seem to support this hypothesis, in fact after 45 min of incubation with 20 nmol of CT, egg phosphatidylcholine/sulfatide liposomes totally retained the entrapped NADH, indicating a size selectivity for the CT induced leakage of molecules in an indicative range between 358 and 709 in molecular weight.

This evidence demonstrates that the interaction of this peptide hormone is able to modify the structural properties of bilayered membranes containing sulfatide, thus promoting an alteration of the barrier properties of the bilayer. It has been demonstrated (Fig. 4) that sulfatide–calcitonin interactions determine an increase in the local concentration of sulfatide in the bilayer. This could alter the molecular packing of lipids at the interphase between the bulk bilayer and the sulfatide enriched domain, thus promoting an increase in CF leakage. An analogous explanation has been proposed by different authors [26–28], to describe the enhanced permeability to CF obtained at the phase

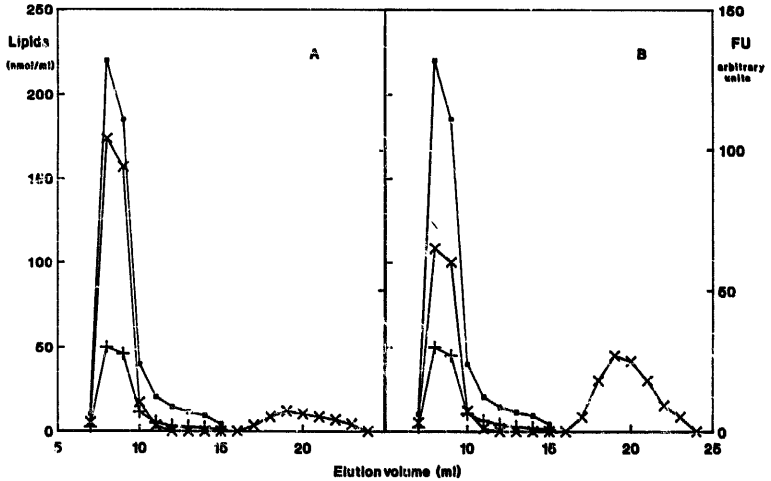

Fig. 7. Elution profiles of egg phosphatidylcholine (■), sulfatide (+) and carboxyfluorescein (×) after chromatography on Sepharose 4B of small unilamellar vesicles of egg phosphatidylcholine/sulfatide (80:20, molar ratio) containing carboxyfluorescein. Before chromatographic separation, vesicles were incubated for 0 (A) and 20 (B) min with calcitonin.

transition temperature of different phospholipid bilayers. Sulfatide–calcitonin interactions might occur in the lipidic microenvironment surrounding the receptor glycoprotein, promoting a modulation of both bioavailability and binding capacity of the receptor itself. On the other hand these interactions might occur in different microenvironments far from the receptor site; also in this case the consequent rearrangement of the membrane organization, could be important in the determination of some physiological activities of the hormone, regardless of the binding of the hormone to the receptor.

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