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Peptide hormone–membrane interactions. Intervesicular transfer of lipophilic gastrin derivatives to artificial membranes and their bioactivities [1]

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Incorporation of di-fatty acylglycerol moieties at the N-terminus of human little-gastrin-(2–17) leads to self-aggregation of the resulting lipo-gastrins into stable, most probably fluid vesicles. Net intervesicular transfer of the lipo-gastrins to phosphatidylcholine model bilayers occurs at high rates whereby the chain length of the gastrin lipid moiety was found to affect the transfer rate more decisively than the nature of the acceptor vesicle. Similarly, the bioactivity of the lipo-gastrins is again affected by the nature of the lipid moiety suggesting differentiated interdigitation with the natural bilayer components and thus, different two-dimensional migration rates to the target receptors. Embedment of the lipo-gastrins in phosphatidylcholine bilayers at high lipid/gastrin ratios as mimicry of the cell membrane bound state does not result in onset of ordered structure, but leads to full exposure of the gastrin in essentially randomly coiled form at the water/lipid interface. This may result from the artificial N-terminal anchorage of the gastrin molecules to the bilayers, but also from the relatively tight packing of the phosphatidylcholine vesicles. Nevertheless, this observation might suggest that in the present case membrane-induced conformation and orientation may not represent a pre-requisite for the hormone receptor binding process. However, the results of this study clearly confirm even for the non-amphiphilic hormone gastrin a membrane-bound pathway for receptor recognition and occupancy.

Introduction

Biologically active peptides which trigger their message via cell membrane-bound receptors, have the possibility of interacting collisionally with biological membranes in the dynamic process of recognition by the receptors and subsequent binding. This interaction of regulatory peptides with membrane surfaces and the resulting membrane-induced conformation and orientation may in fact be considered as a possible pre-requisite for the peptide-receptor binding process, as the stereochemical and functional requirements of the receptor binding site might be better satisfied by this prefolded form than by the random structure usually adopted by unrestrained peptides in the extracellular water phase [1,2]. Therefore, peptide hormones have been increasingly investigated in terms of interaction with model membranes such as liposomes and in this context, their lipo-derivatization is interesting since it should induce or augment their interactions with artificial and natural membranes.

We have recently prepared lipo-derivatives of human little-gastrin, a highly charged linear peptide characterized by the absence of a well expressed primary or secondary amphiphilicity, by synthetizing the adducts of N^{α}-maleoyl-β-alanyl-[Nle^{15}]-human-gastrin-(2–17) with (2*R*,*S*)-1,2-dimyristoyl-3-mercaptoglycerol (DM-gastrin) and (2*R*,*S*)-1,2-dipalmitoyl-3-mercaptoglycerol (DP-gastrin) [3]. The lipo-derivatization has been per-

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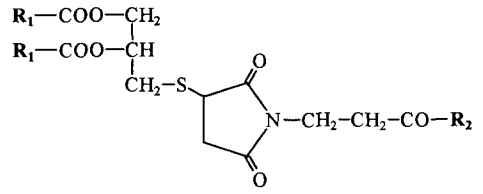
[1] For the first communication of this series see Ref. 3.

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DM-gastrin, dimyristoylmercaptoglycerol/N^{α}-maleoyl-β-alanyl-norleucine-15-little-gastrin-(2–17) adduct; DP-gastrin, dipalmitoylmercaptoglycerol/N^{α}-maleoyl-β-alanyl-norleucine-15-little-gastrin-(2–17) adduct; HG-17, human little-gastrin; hs-DSC, high sensitivity differential scanning calorimetry; CF, 5,6-carboxyfluorescein; CD, circular dichroism.



$R_1 = (CH_2)_{14}-CH_3$ or $(CH_2)_{12}-CH_3$

R_2 = Gly–Pro–Trp–Leu–$(Glu)_5$–Ala–Tyr–Gly–Trp–Nle–Asp–Phe–NH_2

Fig. 1. Structure of the lipo-gastrin derivatives.

formed at the N-terminus of the gastrin, since it is well established that modifications at this position of the hormone molecule do not alter its biological properties [4]. The resulting gastrin derivatives, consisting of a hydrophilic peptide and a hydrophobic lipid portion (Fig. 1), were characterized in respect to their physical and conformational state. The double-tailed lipid moieties were found to induce spontaneous aggregation of the gastrins in aqueous solution to form unilamellar spherical vesicles as well assessed by dynamic light scattering and electron microscopy [3]. Despite their lipid character the gastrin derivatives retain the intrinsic ability to fold into ordered structures similar to those of the parent hormone by lowering the dielectric constant of the medium, e.g., in aqueous-organic solutions, as mimicry of the receptor-bound state.

A spontaneous phospholipid transfer or exchange between phospholipid vesicles has been well established for different model systems by using a wide variety of techniques [5–7]. Therefore, an intervesicular transfer of lipo-gastrin molecules to lipid bilayers is also expected to occur. If this is confirmed, a spontaneous flux of the lipo-gastrins from their own vesicles to the biological membrane should take place as well and thus, lead to a net transfer of these gastrin derivatives to cell surfaces. Hence, the lipophilic derivatization should shift the distribution equilibrium of the hormone molecules in favour of the cell membrane bilayer and correspondingly, generate a depot-type increase in concentration, which in turn could result in an enhanced bioactivity.

On the basis of this working assumption we have investigated the interactions of the lipo-gastrins in their aggregated states with artificial membranes, i.e., with DPPC and DMPC vesicles. These interactions were monitored by changes in the conformational properties of the gastrin component as well as by determining perturbations of the phosphocholine vesicles bilayer. The affinity of the lipo-derivatives for the lipid bilayers has been analyzed following the transfer between lipo-gastrin and phosphocholine vesicles by means of high-sensitivity differential scanning calorimetry (hs-DSC), which provides also precious informations about the bilayer phase behaviour of the pure lipo-gastrins vesicles. Dye leakage experiments have been performed in order to investigate directely the perturbation of the lipo-gastrin vesicles upon addition of DMPC and DPPC. Finally, the potency of these gastrins to stimulate parietal cells was determined in order to correlate their potential accumulation at the cell surface with their bioactivity and thus, to convalidate the hypothesis of a membrane-bound pathway for the receptor recognition and binding process of this peptide hormone.

Materials and Methods

Materials

DMPC, DPPC and 5,6-carboxyfluorescein were purchased from Fluka (Buchs, Switzerland). 3-((3′Ξ)-3′-((2*R*,*S*)-1,2-dimyristoyl-3-thioglyceryl)succinimidyl)propionyl-[Nle^{15}]-HG-(2–17) (= DM-gastrin) and 3-((3′Ξ)-3′-[(2*R*,*S*)-1,2-dipalmitoyl-3-thioglyceryl]succinimidyl}propionyl-[Nle^{15}]-HG-(2–17) (= DP-gastrin) were synthetized and characterized as described previously [3].

Methods

Sample preparation. The peptide concentrations were determined by weight and peptide content of DM-gastrin (88.2% as determined by quantitative amino acid analysis of the acid hydrolysate (6 M HCl, 110°C, 24 h) for M_r 2649.7) and DP-gastrin (89.2%, M_r 2705.3). All buffers were filtered through a 0.2 μm polycarbonate filter (Millipore). Aqueous solutions were prepared by dissolving weighed samples in 0.1% ammonia (5% of the final volume) and diluting with 50 mM Tris adjusted to pH 7.0 with H_3PO_4 unless stated otherwise. In order to obtain homogeneous systems of vesicles, the DM- and DP-gastrin were extruded through a 0.2 μm polycarbonate filter (Nucleopore) according to a known procedure [8] prior to their mixing with DMPC or DPPC at different molar ratios. The same procedure was used for the preparation of DPPC and DMPC vesicles. The concentration of the lipo-gastrins after extrusion was corrected for a dilution factor obtained from the ratio between the absorption spectra of the peptides in the near ultraviolet (UV) before and after extrusion.

High sensitivity differential scanning calorimetry. The lipid transfer from the lipo-gastrin vesicles to the phosphatidylcholine liposomes was investigated with the hs-DSC technique adopting known procedures [9]. The measurements were performed at a scan rate of 45°C/h with an MC-2 (Microcal-Amherst, MA) microcalorimeter interfaced to an IBM AT microcomputer. The data were stored and analyzed using the DA-2 software provided by Microcal. The concentration of the lipo-

gastrins was 1 mg/ml and the peptides to DPPC molar ratio was 1 : 9.

Release of carboxyfluorescein from lipo-gastrin vesicles. The integrity of the lipo-gastrin liposomes was measured by the use of the water soluble fluorescent dye 5,6-carboxyfluorescein (CF) according to a procedure reported previously [10]. CF was entrapped into the liposomes by adding 2 ml of 50 mM Tris adjusted to pH 7.0 with H_3PO_4 and containing 100 mM CF, to the weighed peptide sample. External CF was removed by chromatography of the liposomes over a Sephadex G-50 (superfine) column (1 × 20 cm). The concentration dependent self-quenching of CF allows leakage from vesicles to be monitored continuously. CF fluorescence was measured at an excitation and emission wavelength of 490 and 520 nm, respectively. The fluorescence experiments were performed on a Perkin Elmer 650–40 spectrofluorimeter operating in the ratio mode and equipped with a thermostated cell holder. The measurements were performed by using 2 nm excitation and emission bandwidths. The fluorescence was monitored immediately after mixing the lipo-gastrins vesicles to the phosphatidylcholine liposomes at room temperature and after incubating the samples for 2 h at 50°C. The final peptide concentration was 20 μM.

Circular dichroism. CD spectra were recorded on a Jobin-Yvon dichrograph Mark IV equipped with a thermostated cell holder and connected to a data station for signal averaging and processing. All CD measurements were performed at 20°C and the data averages are of 10 scans. The solutions were incubated for 2 h at 50°C prior to the measurements and were all sufficiently optically clear to allow recording of spectra in the 195–250 nm range. Quartz cells with 0.1 cm optical path were used. The spectra are original computer-drawn CD spectra reported as ellipticity units per mole of amino acid residue ($[\Theta]_R$). The diastereomeric mixture of the 3-{(3′Ξ)-3′-((2*R*,*S*)-1,2-diacyl-3-thioglyceryl)succinimidyl)propionyl moiety does not contribute to the optical activity, since negligible CD was detected in separate measurements on this group as propionic acid derivative [3].

Bioassay. The gastric acid stimulation potency of the lipo-gastrin derivatives was monitored by the [^{14}C] aminopyrine accumulation assay following the procedure recently described [11]. Briefly, rabbit gastric parietal cells were isolated by standard procedures. Cells ($1.5 \cdot 10^6$/ml) were incubated at 37°C for 20 min in Earle's balanced salt solution without bicarbonate (Biomerieux) containing 10 mM Hepes and 0.2% BSA (pH 7.4), with 0.05 μCi [^{14}C]aminopyrine and [Nle^{15}]-HG-17 and the lipo-gastrins (final concentration range: 10^{-6}–10^{-11} M); 1 mM stock solutions of gastrin and lipo-gastrins were prepared in 1 mM NH_4OH. [^{14}C] Aminopyrine accumulation was determined by measuring the percent of radioactivity recovered in the cell pellet and was expressed as percentage over the basal value. The accumulation of [^{14}C]aminopyrine in the presence of 10 mM NaSCN was considered as non-specific uptake and subtracted from the assay values.

Results and Discussion

Lipid transfer between lipo-gastrin and phosphatidylcholine vesicles

By hs-DSC measurements a chain melting transition between 3° and 65°C could not be observed for both the pure DP- and the DM-gastrin vesicles. This suggests that the vesicles are in the liquid state above 3°C despite the double-tailing of the lipid moiety with C_{16}- and C_{14}-fatty acids. This remarkable fluidification of the bilayers can be explained by the relatively large, negatively charged (six carboxylates) hydrophilic peptide chain, promoting the formation of very small vesicles. The small size of the gastrin vesicles becomes obvious from the near perfect transparency of the vesicle solution used for these experiments. It is well known that such vesicles exhibit low cooperative phase transitions at significantly lower transition temperatures [12]. Hence, it is more likely that our gastrin vesicles are in the fluid state rather than in a highly solved gel state. By ^{2}H-NMR employing chain perdeuterated (C_{16})-lipogastrin incorporated into a DPPC bilayer, we observed that the phase state of the peptide di-fatty acyl moiety is determined by the host lipid phase state (details of this study will be reported elsewhere).

It has been well established that the relative fluidity of the donor vesicles is much more important than that of the acceptor vesicles in intervesicular lipid transfer processes [13]. Therefore, a low phase transition temperatures of the two lipo-gastrins should facilitate their transfer to phosphatidylcholine vesicles. In fact, the DM-gastrin transfer to DPPC liposomes at a DM-gastrin/DPPC molar ratio of 1 : 9 was found to proceed at high rates as shown in Fig. 2. The hs-DSC endotherm of the system right after mixing shows that the gel to liquid-crystal transition curve of the DPPC vesicles population has already been affected by the DM-gastrin transfer which leads to suppression of the pre-transition peak at 35.3°C that is characteristic for pure DPPC vesicles. With increasing incubation time, the initial sharp peak at 40.5°C rapidly broadens to give a large peak of low intensity at 40.6°C after an incubation time of 1.5 h at the average temperature of 50°C. Upon an additional incubation of 6 days at room temperature, the hs-DSC trace of the mixture presented the same broad peak but at sensibly higher transition temperature (43.2°C) which can be reasonably explained by the presence of DPPC and DM-

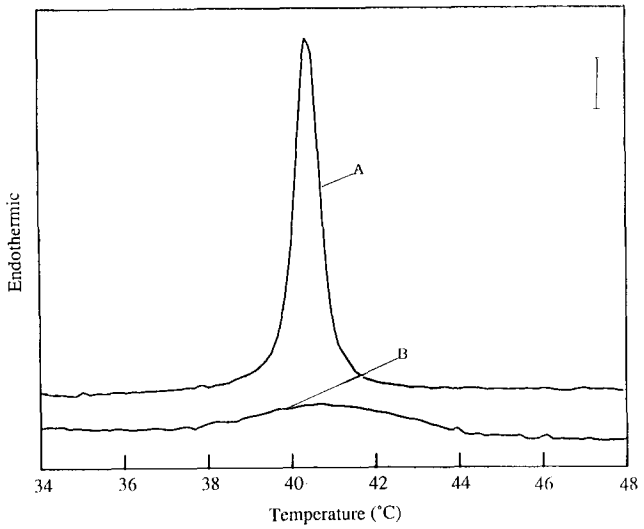


Fig. 2. Hs-DSC endotherms of a 1:9 (mol) mixture of two vesicle populations of DM-gastrin and DPPC prepared by extrusion (0.2 μm) at an incubation time $t = 0$ (A) and $t = 1.5$ h (B) at an average temperature of 50°C. The peptide concentration was $3.4 \cdot 10^{-4}$ M. The bar corresponds to 1 kcal/deg per mol.

gastrin hydrolytic breakdown products in the mixture [12].

When the transfer of DP-gastrin from its vesicles to DPPC liposomes has been investigated, the hs-DSC experiments showed that the transfer process with DP-gastrin at a 1:9 DP-gastrin/DPPC molar ratio occurs at a significantly lower rate than that observed for DM-gastrin. A continuous reduction of the peak intensity reaches a constant pattern after 7 h of incubation above the transition temperature of DPPC as shown in Fig. 3. Although the peak remains sharp with minor effects on the transition temperature, this does not exclude that a net transfer of DP-gastrin has occurred. The low effect of the transferred DP-gastrin on the transition temperature of the acceptor DPPC bilayer may result from the identical lipid moieties. In this context it is worthy to note that even the net transfer of DM-gastrin, although broadening the transition peak, was not found to affect significantly the transition temperature of the acceptor DPPC vesicles. Upon an additional incubation period of six days the endotherm of the DP-gastrin/DPPC system did not look significantly different in size and shape from the previous hs-DSC trace except for the transition temperature which, as already observed in the DM-gastrin experiment, was shifted to a higher value (42.4°C).

From previous studies it is known that the rate of lipid transfer depends exponentially on the acyl chain length, e.g., the K_{off} of DMPC is 20-fold higher than the K_{off} of DPPC [14], and that irrespective of the degree of fluidity of the donor and acceptor vesicle the rate of transfer of dimyristoyl-lipids is higher than that of dipalmitoyl-lipids [12]. Thus, the observed lower rate of DP-gastrin transfer in comparison to that of the DM-g astrin transfer to DPPC fully agrees with previous observations despite the low phase transition temperatures of both type of vesicles.

The mechanism of monomer transfer between bilayer vesicles through the bulk solution implies a transfer rate that depends upon both the molecular structure and the characteristics of the donor and acceptor bilayers [7]. Our results suggest that the process of lipo-gastrin transfer, although related to a single acceptor vesicle population, i.e., the DPPC liposomes, is mainly dictated by the packing of the donor vesicle. We have therefore analyzed the effect of a possibly different packing of the lipid moieties on the conformational properties of the gastrin chains and the results clearly indicate a tighter packing of the DP-gastrin in comparison to the DM-gastrin in the aggregated state (see below).

Dye leakage of lipo-gastrin vesicles upon interaction with phosphatidylcholine liposomes

Merging of the lipo-gastrins with artificial membranes as model for the accumulation of these gastrin derivatives in cell membrane was further examined by carboxyfluorescein leakage from DM- as well as DP-gastrin vesicles at increasing lipid/lipo-gastrin ratios. These dye leakage experiments allowed us to monitor more precisely the perturbation of lipo-gastrin vesicles upon DPPC or DMPC addition, although this type of experiments is not directly related to the monomer transfer process itself, since vesicle disrupture and dye release may also result from collisional events. The results reported in Figs. 4 and 5 indicate that disrup-

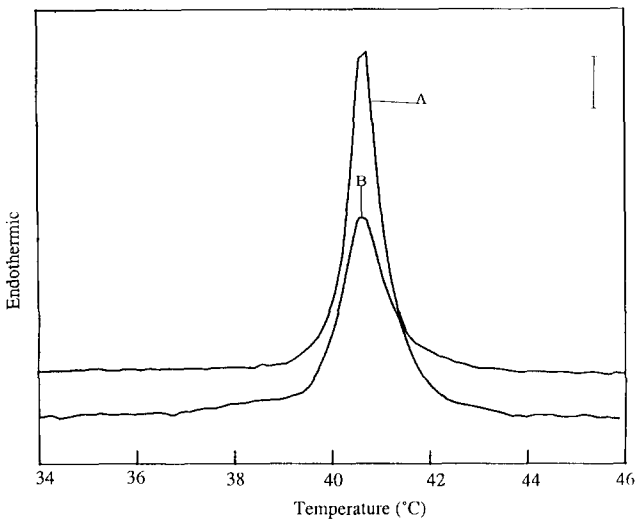


Fig. 3. Hs-DSC endotherms of 1:9 (mol) mixture of two vesicle populations of DP-gastrin and DPPC prepared by extrusion (0.2 μm) at an incubation time $t = 0$ (A) and $t = 7$ h (B) at an average temperature of 50°C. The peptide concentration was $2.9 \cdot 10^{-4}$ M. The bar corresponds to 1 kcal/deg per mol.

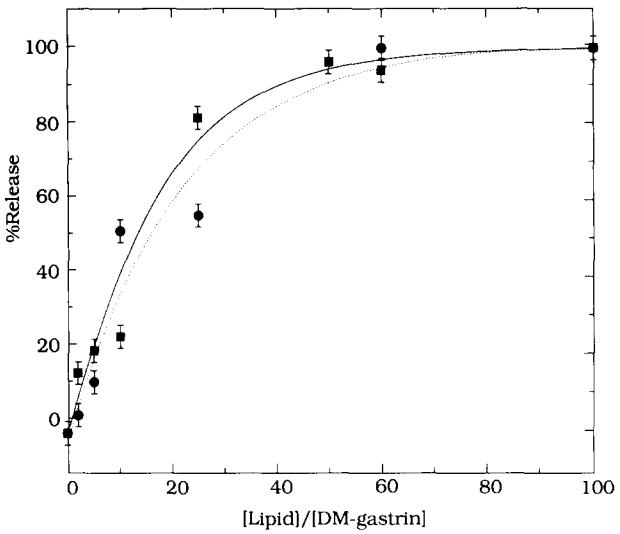


Fig. 4. Carboxyfluorescein leakage from DM-gastrin vesicles as a function of DPPC/DM-gastrin (■ ——— ■) and DMPC/DM-gastrin (● · · · · · ●) molar ratio. Dye leakage was measured after 2 h incubation at 50°C and is expressed as percentage of the plateau fluorescence value. The peptide concentration range was $3 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$ M. The relative error in percentage CF release amounts to 3% as determined by small error analysis.

ture of lipo-gastrin vesicles occurs more easily with DM-gastrin than with DP-gastrin. In the first case a peptide/phosphatidylcholine molar ratio of 1:50 is required for a maximum fluorophore release while the same result is obtained with DP-gastrin only at a molar ratio of 1:100. Interestingly, the leakage seems to occur very fast since a time dependent significant increase of the fluorescence of the medium could not be detected upon incubation of the mixed samples at 50°C, i.e., above the phase transition temperature of DMPC and DPPC. Furthermore, a comparison of the leakage measured for each lipo-gastrin derivative in the presence of DMPC with that monitored in presence of DPPC excludes a major role of the acceptor vesicle population in the disrupture process of the lipo-gastrins vesicles since the corresponding correlation curves are very similar as shown in Figs. 4 and 5. Consequently, the disrupture of the lipo-gastrin bilayers is mainly conditioned by their own lipid packing and thus, by the nature of the di-fatty acyl moieties.

Conformational properties of the lipo-gastrins embedded in phosphatidylcholine bilayers

The CD spectra of DP- and DM-gastrin in Tris adjusted to pH 7.0 with H_3PO_4 are reported in Fig. 6. The spectrum of DP-gastrin exhibits a large negative band centered at 204 nm with a broad shoulder in the 215–220 nm range. Conversely, the spectrum of DM-gastrin although characterized by the identical negative maximum of equal dichroic intensity at 204 nm, exhibits an additional negative band centered at 215–218 nm of remarkably higher intensity than the shoulder observed in the former spectrum. This enhanced CD in the $n - \pi^*$ transition range clearly reveals for DM-gastrin vesicles the presence of ordered structure at a sensibly higher degree than for DP-gastrin. This difference in the content of secondary structure of the gastrin moiety should result from altered environments. Previous conformational studies on gastrin and gastrin-related peptides in aqueous and aqueous-organic media have shown that the linear flexible gastrin molecule is essentially randomly coiled in water, but that by lowering the dielectric constant of the medium, e.g., in aqueous trifluoroethanol solutions,

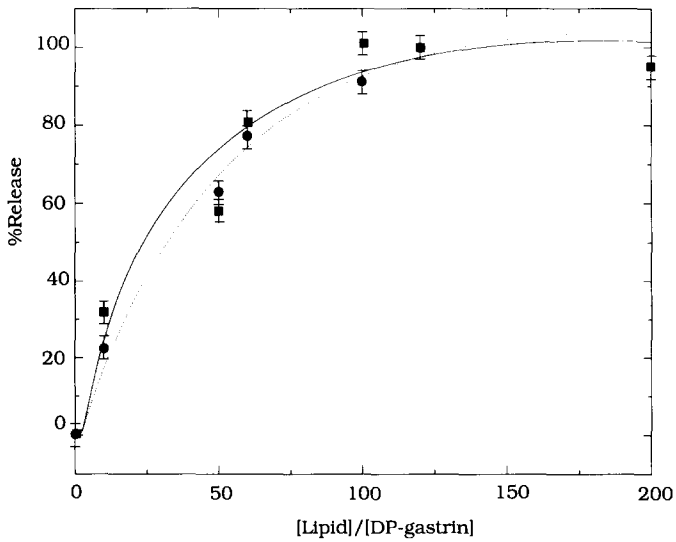


Fig. 5. Carboxyfluorescein leakage from DP-gastrin vesicles as a function of DPPC/DP-gastrin (■ ——— ■) and DMPC/DP-gastrin (● · · · · · ●) molar ratio. Dye leakage was measured after 2 h incubation at 50°C and is expressed as percentage of the plateau fluorescence value. The peptide concentration range was $3 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$ M. The relative error in percentage CF release amounts to 3% as determined by small error analysis.

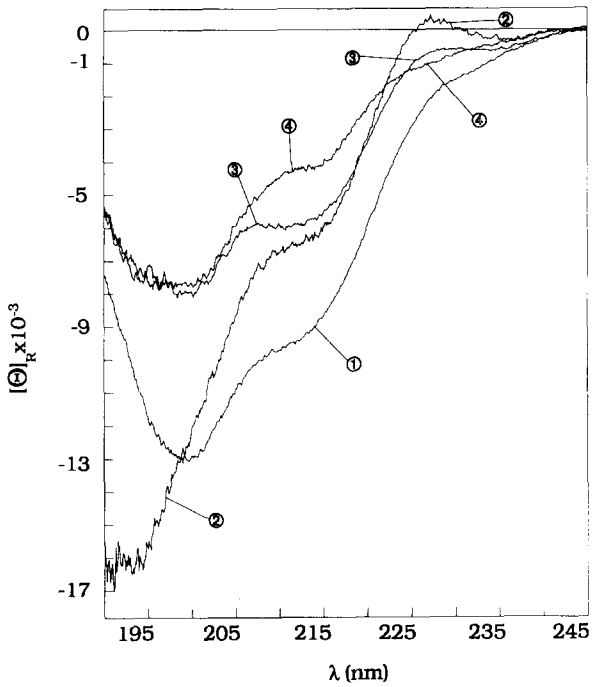


Fig. 6. CD spectra of DM-gastrin in 5 mM phosphate buffer (pH 7.0) (curve 1), 5 mM phosphate buffer containing 100 mM NaCl (pH 7.0) (curve 2), and 50 mM Tris adjusted to pH 7.0 with H_3PO_4 (curve 3). The spectrum of DP-gastrin in 50 mM Tris adjusted to pH 7.0 with H_3PO_4 has been reported for comparison (curve 4); peptide concentrations are $3 \cdot 10^{-5}$ M.

highly ordered structures are induced [15–17]. An altered, more hydrophobic environment of the gastrin chain in DM-gastrin vesicles in respect to DP-gastrin vesicles could result from a weaker packing of the dimyristoylglycerol in comparison to that of the dipalmitoylglycerol moiety that leads to an enhanced interaction of the peptide chain with more hydrophobic compartments of the vesicle bilayer. This weaker packing of the DM-gastrin vesicles in comparison to the DP-gastrin may also be responsible for the faster transfer of the DM-gastrin in respect to the DP-gastrin to DPPC as monitored in the hs-DSC experiments described above.

The dichroic properties of the peptide moiety in the lipo-gastrin vesicles are also affected in significant manner by the ionic strength of the buffer as shown in Fig. 6. The ionic strength of the medium is known to influence the bilayer packing together with the nature of the di-fatty acyl moieties. Tighter packing as induced by high ionic strength is, in fact, exposing the gastrin chains to the aqueous phase, thus inducing predominantly random coil structure, the typical conformational state of gastrin in aqueous solution [15].

Embedment of the lipo-gastrin derivatives into artificial membranes has been followed by monitoring the changes in the dichroic properties of the gastrin moiety at different lipid/DM-gastrin molar ratios. Incorporation of lipo-gastrin into the zwitterionic phosphatidylcholine bilayers leads in ratio dependency to strong changes of the conformational properties of the gastrin moiety. As shown in Fig. 7 the negative band at 215–218 nm as index of ordered structure disappears upon increasing the DPPC content. The negative band at 204 nm is blue-shifted to 200 nm with concomitant increase in intensity up to a DPPC/DM-gastrin molar ratio of 10:1; then the intensity of this band weakens to reach half of its maximum value at the ratio of 50:1. Thereby, the overall CD pattern with the negative band centered at 200 nm and the positive band at 228 nm assumes the typical shape for absence of ordered structure to major extents. Thus at high lipid/gastrin ratios the tight packing of the DPPC component apparently becomes dominant leading to exposure of the gastrin chains to the aqueous environment to a degree which is higher than that observed in DP-gastrin vesicles as can be deduced by comparing the CD spectra of DP-gastrin (Fig. 6) and of DPPC/DM-gastrin (50:1) (Fig. 7). Alternatively, these dichroic changes could also be attributed to a dilution effect on the conformational properties of the peptide chains upon merging the vesicles, if gastrin–gastrin interactions resulting from the high peptide density at the lipo-gastrin bilayer surface is significantly contributing the onset of ordered structure. However, aggregation of gastrin peptides and thus enhanced gastrin-gastrin interactions has previously been found to generate CD spectra characteristic for β-structure and thus, very dissimilar

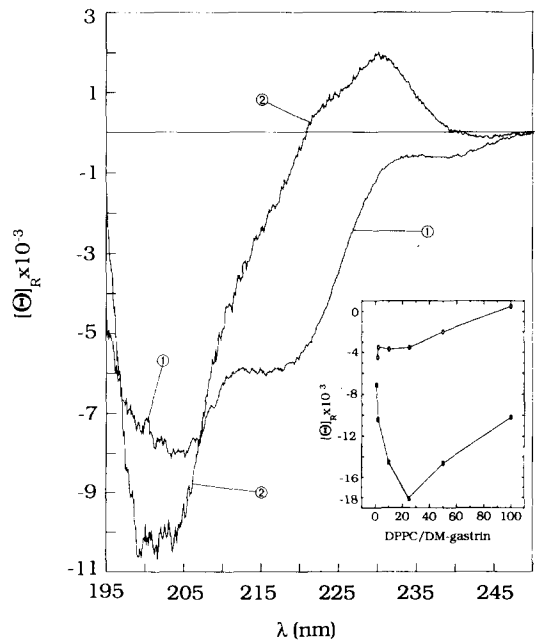


Fig. 7. CD spectra of DM-gastrin in 50 mM Tris adjusted to pH 7.0 with H_3PO_4 at a peptide/DPPC molar ratio of 1:0 (curve 1) and 1:50 (curve 2); peptide concentration was $3 \cdot 10^{-5}$ M. Inset: plot of $[\Theta]_{200}$ (■ ——— ■) and of $[\Theta]_{222}$ (● ——— ●) vs. DPPC/DM-gastrin molar ratio.

from those monitored in the present case for the lipo-gastrins [18]. This seems to support a dominant role of the lipid components in the conformational behaviour of the gastrin portions.

As expected from this working assumption, upon embedment of DM-gastrin into DMPC the negative band at 215–218 nm is retained up to a DMPC/DM-gastrin ratio of 25 : 1 (see Fig. 8). Although the negative band at 204 nm is slightly blue-shifted and of increased intensity, the CD properties indicate that the ordered structure of the gastrin chain as present in the DM-gastrin vesicles is largely retained. Only upon a further increase of the DMPC content to ratios of 50 : 1 and 100 : 1, a collapse of the gastrin ordered structure occurs as well assessed by the corresponding CD spectra where the band at 215–218 nm is reduced to a very weak shoulder and the overall CD pattern is indicating the absence of ordered structure at significant extents. A similar monitoring of the conformational effects of DPPC and DMPC on DP-gastrin could not be performed since a recording of reliable CD spectra for this system was prevented by the strong turbidity of the test samples at high DPPC/peptide ratio.

The conformational effects observed upon incorporation of lipo-gastrin DPPC and DMPC into the vesicles have to be attributed to changes induced in the peptide environment by the continuous increase of DPPC and DMPC head groups surrounding the inserted lipo-gastrin, but also to different bilayer packings which again result in altered gastrin environments.

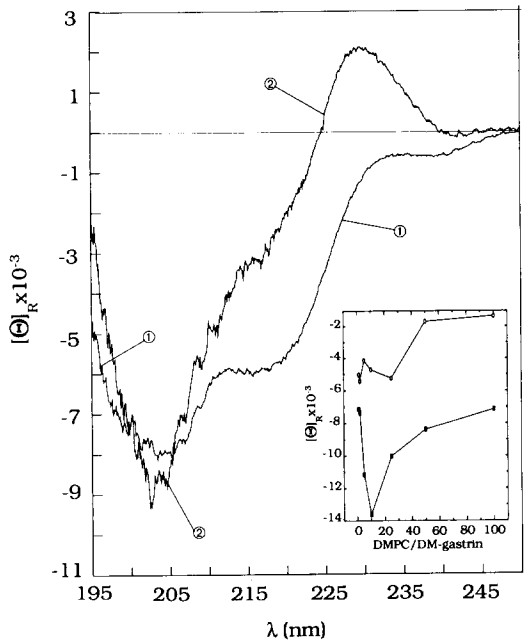


Fig. 8. CD spectra of DM-gastrin in 50 mM Tris adjusted to pH 7.0 with H_3PO_4 at a peptide/DMPC molar ratio of 1:0 (curve 1) and 1:100 (curve 2); peptide concentration was $3 \cdot 10^{-5}$ M. Inset: plot of $[\Theta]_{200}$ (■——■) and of $[\Theta]_{222}$ (●——●) vs. DMPC/DM-gastrin molar ratio.

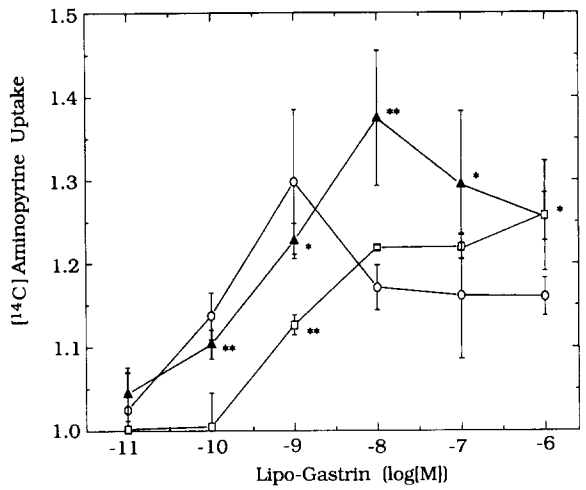


Fig. 9. Gastric parietal cells ($2 \cdot 10^6$/ml) were incubated with gastrin ([Nle^{15}]-HG-17), DM-gastrin and DP-gastrin at various concentrations in standard medium with 0.05 μCi [^{14}C]aminopyrine for 20 min at 37°C under continuous gassing (O_2/CO_2). Basal [^{14}C]aminopyrine uptake was 5.5 + 0.3%. [Nle^{15}]-HG-17 (○——○); DM-gastrin (▲——▲) and DP-gastrin (□——□). Statistical significance was determined using Student's *t*-test. * $P < 0.05$ and ** $P < 0.01$, significantly different from the value observed for gastrin.

The continuous change of the dichroic properties brought about by the increased lipid/lipo-gastrin ratio should also confirm that an embedment of the lipo-gastrin into acceptor membranes does not occur in clusters, but in statistical distribution over the acceptor bilayer. This in turn would agree more properly with the mechanism of monomer transfer via the bulk solution than with that of vesicle fusion upon collision.

Bioactivity of lipo-gastrins

Despite the spontaneous aggregation of the lipo-gastrin derivatives into surprisingly stable vesicles, these were found to retain remarkable gastrin receptor affinities. For DM-gastrin a 7-fold and for DP-gastrin an 11-fold weaker binding than that of the parent gastrin hormone was determined in gastrin-tracer displacement experiments from AR4–2J cell membrane preparations known to contain high affinity gastrin/CCK receptors [3]. The biological activities of the two lipo-gastrins were evaluated by their ability to induce aminopyrine uptake as index for acid secretion in isolated parietal cells of the rabbit ($n = 6$ separate experiments). As shown in Fig. 9 the response to gastrin ([Nle^{15}]-HG-17) was dose-dependent with a maximal stimulation at 1nM and with an EC_{50} of 0.2 nM. As previously observed [19] this response was biphasic with a decrease at 10 nM gastrin concentration. DM- and DP-gastrin were compared in this experimental model to gastrin. DM-gastrin was found to be slightly more efficient but less potent (5-fold) than the parent gastrin. The maximal effect was observed at 10 nM and the dose response curve was found to be again biphasic

with a decrease at concentrations higher than 100 nM. DP-gastrin exhibited an efficacy similar to that of gastrin, but it proved to be less potent than DM-gastrin (1.5-fold) whereby the maximal stimulation was observed at 50 nM without any decrease in this biological activity up to the concentrations examined. Although the binding affinities of the lipo-gastrins for the gastrin/CCK receptors of AR4–2J membrane preparations and their potencies in stimulating acid release in parietal cells cannot be directly correlated because of the different cell types, a similar pattern is observed in respect to the parent gastrin hormone.

The bioassays were performed in the 10^{-6} to 10^{-11} M concentration range, i.e., mainly at lower concentrations than those used for the spectroscopic measurements which confirmed aggregation of the lipo-gastrins into vesicles. We were unable to determine the critical bilayer concentration of the lipo-derivatives and thus, their aggregational state at high dilutions. However, the patterns of the dose-response curves of the two lipo-gastrins are, within the limits of error of the assay, similar to that of the monomeric parent hormone although shifted to lower potencies. Thus, irrespective of the physical state of the lipo-gastrins at high dilution, i.e., vesicles, micelles or monomers, collisional events with the cells leads to transfer of the lipo-peptides to the membrane bilayer. The model studies on transfer of the lipo-gastrins to phosphatidylcholine vesicles, described above, clearly indicated that this process is occurring at lower rates for DP-gastrin than for DM-gastrin vesicles. The lower potency of the DP-derivative than that of the DM-gastrin fully agrees with the results of the model experiments and it may therefore be attributed to the transfer process, but on a similar rationale also to a lower rate of two-dimensional diffusion to the target receptor as resulting from a tighter interdigitation of its di-fatty acyl moiety with the lipids of the cell membrane bilayer. Despite the fluidity of the natural membranes and correspondingly, the relative fast movements of single components, the chain lenght of the fatty acids may strongly affect even this migration rate.

Conclusion

Both our previous studies on the interaction of lipo-gastrins with surfactants [3] and more properly the present investigation on the lipo-gastrin/phosphatidylcholine system indicate that even an induced lipid interaction does not result in onset of ordered structure for the gastrin molecule at significant extents, when high lipid/gastrin ratios are used to mimic a cell membrane-bound state of the hormone. Schwyzer et al. [20] have recently reported that the unmodified highly hydrophilic gastrin molecule is structured into α-helix upon its incorporation into lipid multilayer films as well assessed by infrared-spectroscopy in the attenuated total reflection mode. These different results may be attributed to the different systems used, i.e., embedment of gastrin into lipid films vs. anchorage of gastrin to bilayer surfaces; but it may also derive from the specific orientation induced in our case via the N-terminal lipo-derivatization. However, irrespective of whether the gastrin molecule is prefolded or not upon collision with the cell membrane, the mechanism of receptor occupancy of hormones via their accumulation at the membrane surface and subsequent two-dimensional migration to the target receptor was confirmed even for the non-amphiphilic gastrin molecule as induced lipid interaction was not found to prevent the hormonal activity, whereas the chain lengths of the lipid moiety do affect the potency.

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