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Structural properties of acidic phospholipids in complexes with calcitonin: a Fourier transform infrared spectroscopic investigation *

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The interaction of the polypeptide hormone calcitonin with two acidic phospholipids, dimyristoylphosphatidylglycerol (DMPG) and dimyristoylphosphatidic acid (DMPA), was investigated by Fourier-transform infrared spectroscopy. The association of calcitonin with DMPG results in a broadening of the lipid phase transition, accompanied by a marked decrease in the conformational order of the acyl chains at temperatures below the phase transition region. Infrared bands due to carbonyl ester and phosphate group vibrations of DMPG molecules are not significantly affected by the presence of calcitonin. The effect of calcitonin on the conformation of acyl chains in DMPA is much smaller compared with DMPG. The different susceptibility of DMPG and DMPA to perturbation by calcitonin is suggested to be related to different degrees of intermolecular interactions between the headgroups of these two phospholipids.

Calcitonin is a 32 amino acid polypeptide hormone which has a high affinity for certain acidic phospholipids [1]. As with some other biologically important peptides [2,3], the structural feature which facilitates the interaction of calcitonin with lipids is its ability to fold into an amphipathic helix [1]. The growing interest in the interaction between polypeptides forming amphipathic helices and phospholipids is stimulated by indications that similar interactions can contribute to the binding of certain hormones to cell surface receptors [2–5]. The understanding of various aspects of this interaction is also of more general importance from the point of view of the mechanism of protein insertion into the lipid bilayer and the structural properties of lipoprotein complexes. In previous model studies, salmon calcitonin has been shown to solubilize multilamellar liposomes of certain acidic phospholipids to form small particles of a morphology similar to that found in recombinants formed between phospholipids and certain serum apolipoproteins [1,6]. The effect of this interaction on the conformational properties of lipid molecules remains, however, largely unknown.

In this communication we report the results of a Fourier transform infrared (FT-IR) spectroscopic investigation of the interaction between salmon calcitonin and the acidic phospholipids dimyristoylphosphatidylglycerol (DMPG) and dimyristoylphosphatidic acid (DMPA). The advantage of infrared spectrometry is that, without introducing perturbing probe molecules, both the packing and conformation of phospholipid acyl

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chains and the structure of the polar regions can be probed via the characteristic vibrations of specific chemical groups [7–9].

The carbon-hydrogen stretching vibrations of phospholipid acyl chains give rise to a number of well defined bands in the 3100–2800 cm^{-1} region of the infrared spectrum. The vibration most suitable for following lipid structural changes in the presence of proteins is the CH_2 symmetric mode near 2850 cm^{-1}. This vibration is essentially free of contribution from the protein component and it is very sensitive to the conformation of lipid acyl chains. In particular, the frequency of the above infrared mode responds in a regular manner to changes in the *trans/gauche* ratio in lipid acyl chains, and it can be used as a measure of lipid conformational order [7–9].

Fig. 1 shows frequency vs. temperature plots of the CH_2 symmetric vibrations for DMPG alone and DMPG in the presence of calcitonin at two different concentrations. At the lipid and peptide concentrations used it is expected that essentially all of the peptide is bound to the lipid over a wide range of temperatures [1]. The sharp discontinuity of the plot for pure lipid at 24°C reflects the cooperative gel-liquid crystalline phase transition. The DMPG-calcitonin complexes exhibit significant differences from the control system. First, in the presence of the peptide the temperature range of the lipid melting is considerable broadened and the midpoint of the transition is shifted to slightly higher temperatures. This is in agreement with previous results from differential scanning calorimetery [1]. Second, the physical state of the acyl chains at temperatures beyond the transition region is perturbed by the peptide in a concentration-dependent manner. At temperatures below the phase transition the frequencies of the CH_2 symmetric stretching vibrations are shifted to considerable higher values. This effect becomes stronger as the peptide to lipid molar ratio is increased. On the other hand, at temperatures above the melting region no significant changes in frequency are observed at a peptide to lipid molar ratio of 1 : 20 and only a small frequency increase is seen at a peptide to lipid molar ratio of 1 : 10. From these data it may be concluded that upon complexation with calcitonin the conformational order of DMPG molecules below the transition

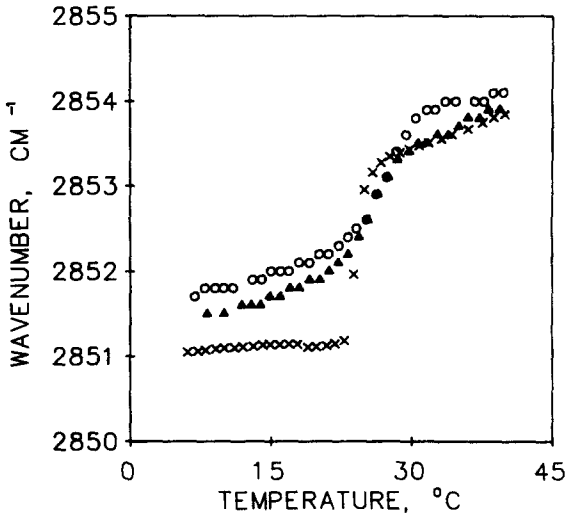

Fig. 1. Temperature dependence of the position of the CH_2 symmetric stretching band in pure DMPG multibilayers (×) and in DMPG complexed with salmon calcitonin at the lipid to peptide molar ratio of 20 : 1 (▲) and 10 : 1 (○). Lipid-peptide complexes were prepared by adding calcitonin solution in buffer (50 mM Hepes (pH 7.0) prepared in ^2H_2O or H_2O) to a lipid powder. The mixture was vortexed for approx. 5 min during which time the sample was warmed and cooled repeatedly through the transition temperature. The final lipid concentration was approx. 100 mg/ml. Infrared spectra were measured between BaF_2 windows that were assembled into a demountable liquid cell (Harrick Scientific, Ossining, NY), using 12 μm spacers. FT-IR spectra were recorded using a Digilab FTS-15 instrument equipped with a HgCdTe detector. For each spectrum, 250 interferograms were collected, coadded, apodized with a triangular function and Fourier-transformed to give a resolution of 2 cm^{-1}. Temperature was controlled by the computer and during data acquisition it was stable within 0.1 Cdeg. The average heating rate was 5 Cdeg/h. Frequencies were determined with an accuracy of ±0.01 cm^{-1} by using a center of gravity algorithm [22]. Phosphilipids were obtained from Avanti Polar Lipids, Inc. Salmon calcitonin was synthetized by Armour Pharmaceutical Co., Kankakee, IL.

temperature is considerably reduced as a result of the formation of additional *gauche* rotamers. It is worth mentioning that the observed conformational disordering represents the average effect of the peptide on the DMPG acyl chains. The present infrared experiments cannot distinguish whether calcitonin disorders all of the lipid molecules or whether binding of the peptide results in more than one population of lipid with different conformational ordering. Above the transition temperature the conformational order of lipid acyl

chains in the complex is similar to that in pure DMPG, becoming sightly reduced only at a high concentration of the peptide.

The increased conformational disorder of the acyl chains of gel phase DMPG complexed with calcitonin closely resembles the behaviour of DMPC in complexes with another amphipathic helix-forming polypeptide, glucagon [10]. This disording of the gel state may explain why lipid complexes of both calcitonin [1] and glucagon [11] have a lower transition enthalphy than that of the pure lipid. The mechanism of the interaction of these two peptide hormones with phospholipids is, however, not identical. Glucagon interacts equally well with zwitterionic and acidic phospholipids, whereas calcitonin shows a strong preference for the latter [12]. Moreover, in contrast to the glucagon-DMPC complex which dissociates above the phase transition of the lipid [13], the calcitonin-DMPG complex is stable over a wide range of temperatures. It appears, however, that although calcitonin is still bound to DMPG in the liquid-crystalline state, the peptide no longer affects the conformational order of the acyl chains. This may be interpreted to suggest that the peptide has a greater solubility in the hydrophobic core of the lipid bilayer when the lipid is in the gel state than when it is in the liquid-crystalline state. In analogy with Raoult's law [14], we would then predict that calcitonin will raise the phase transition temperature of DMPG as is observed experimentally.

The apparent specificity of the positively-charged calcitonin for acidic phospholipids suggests the involement of electrostatic interactions. This prompted us to investigate the effect of calcitonin on the polar region of DMPG. Infrared bands characteristic for this part of the phospholipid molecules are the ester C=O stretching vibration in the 1750–1700 cm^{-1} region, and the phosphate group vibrations between 1300 and 1000 cm^{-1}. However, these bands were found to be essentially unaffected by calcitonin (spectra not shown). This suggests that the interaction of calcitonin with DMPG does not result in a formation of a strong peptide-lipid headgroup complex in which the electronic environments of either phosphate or carbonyl groups are altered. Also, significant dehydration of the headgroup region in the presence of calcitonin appears to be unlikely. Both of these effects would lead to shifts and/or splittings of the phosphate and carbonyl bands, as was observed for example upon complexation of phosphatidylserine with calcium or lithium ions [15,16]. On the other hand, the possibility of small changes in the mobility of DMPG headgroup cannot be excluded since the infrared bands due to both phosphate and carbonyl groups are not very sensitive to subtle changes in the mobility of these moieties. Thus, although the electrostatic forces between the peptide and phospholipid headgroups may play an important role in the initial absorption of calcitonin onto the membrane surface and in providing favourable conditions for its subsequent insertion into the bilayer interior, it seems that the interaction in the already formed lipoprotein particles is predominantly of a hydrophobic nature. Another factor contributing to the much stronger ability of calcitonin to form lipoprotein particles with DMPG than with DMPC may be the less compact structure of the acidic DMPG bilayer resulting from the repulsive forces between negatively charged headgroups. A similar mechanism may be also operative in the recently observed preferential interaction of apolipoprotein A-I with acidic phospholipids [17].

Experiments similar to those described for DMPG have been performed also with another acidic phospholipid, DMPA. As shown in Fig. 2 in this case calcitonin has only a very small, if any, effect on the temperature dependence of the CH_2-stretching frequency. The much smaller perturbation by calcitonin of the molecular conformation of acyl chains of DMPA is in accord with the observation that calcitonin is less lytic to phosphatidic acid-containing vesicles than to vesicles with DMPG [6]. In addition, the morphology of complexes formed between calcitonin and DMPA is different from that with DMPG [6]. As both phospholipids have the same hydrocarbon chains and at the experimental pH they possess a single negative charge [18], the difference in their ability to interact with calcitonin must be due to the different intermolecular interactions in the polar headgroup region. Numerous lines of evidence indicate that phosphatidic acid, but not phosphatidylglycerol, interacts intermolecularly via hydrogen bonding between adjacent polar headgroups [18–20]. The strong intermolecular hy-

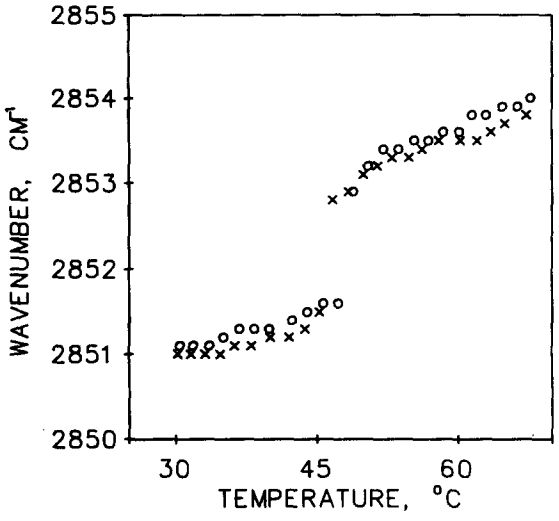

Fig. 2. Temperature dependence of the position of the CH_2 symmetric stretching band in pure DMPA multibilayers (×) and DMPA in the presence of salmon calcitonin at a lipid to protein molar ratio of 10:1 (○). Experimental conditions as those given in legend to Fig. 1.

drogen bonding, combined with a smaller size of the polar headgroups of phosphatidic acid are likely to result in much tighter packing of DMPA compared to that of DMPG. The tight packing of DMPA headgroups may hinder the insertion of calcitonin into the bilayer interior, thus preventing hydrophobic association of the peptide with the lipid acyl chains. A similar role of interlipidic hydrogen bonding has been recently proposed to explain the differences in the penetration of pentagastrin related pentapeptides into the bilayers of different phospholipids [21].

In conclusion, the present FT-IR experiments show that the association of calcitonin with DMPG molecules results in a considerable reduction of the conformational order of lipid acyl chains at temperatures below the lipid phase transition. The conformation of the polar regions of DMPG does not seem to be affected significantly by the presence of calcitonin. The effect of calcitonin on the acyl chain conformation of the intermolecularly hydrogen-bonded DMPA is much weaker than it is on the acyl chain conformation of DMPG.

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