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Effect of the antitumour protein α-sarcin on the thermotropic behaviour of acid phospholipid vesicles

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The antitumour protein α-sarcin modifies the thermotropic behaviour of phospholipid vesicles. This has been studied by fluorescence depolarization measurements and differential scanning calorimetry. A surface protein–phospholipid interaction is detected by measuring the polarization degree of TMA-DPH-labelled vesicles. At the higher protein / lipid molar ratios studied, the α-sarcin-vesicles complexes exhibit different thermotropic behaviour depending on whether they are prepared above or below the T_m of the corresponding phospholipid. Labelling of the protein with photoactive phospholipids has also been considered. α-Sarcin penetrates the bilayer deep enough to be labelled with the photoactive group located at the C-12 of the fatty acid acyl chain of phospholipids forming vesicles.

Introduction

α-Sarcin is a cytotoxic protein produced by the mould *Aspergillus giganteus*. This molecule behaves as antitumour agent since it inactivates the larger subunit of ribosomes from some tumour and virus-infected cells [1,2]. Such an inactivation is due to the highly specific ribonuclease activity exhibited by α-sarcin (see Ref. 3 for a review). The antitumour character of α-sarcin also requires the ability of the protein to enter the target cells, which is under current study. α-Sarcin, a single polypeptide chain protein, produces aggregation of phospholipid vesicles and exhibits specific requirements for acid lipids [4]. The protein also promotes fusion of lipid bilayers [5]. Divalent cations do not seem to be involved on the observed lipid–protein interactions [6]. Thus, these significant alterations produced by α-sarcin in bilayers could be related to its antitumour character.

The molecular mechanisms underlying membrane fusion are not well known. It is obvious that structural reorganizations of the lipid arrangement have to occur during fusion. The most prominent thermodynamical feature related to the arrangement of the lipid bilayers is their characteristic gel-to-liquid crystalline transition. This phase transition arises from the cooperative melting of the acyl chains of the lipid molecules. This process is dependent on the length and composition of the acyl chain as well as on the characteristics of the polar headgroup of the phospholipid. For a particular type of lipid molecule, this transition can be modified by extrinsic factors such as pH and ionic strength, and also interacting proteins. The modification performed by a protein on the thermal transition of a bilayer would give information about the molecular process inherent to the lipid–protein interaction. Thus, the analysis of the cooperative melting of the hydrocarbon chains in lipid bilayers has been revealed as a very useful tool for the study of protein–lipid interactions. So, the effect of α-sarcin on the thermotropic behaviour of lipid model systems has been considered in order to get new insights about its antitumour character.

Materials and Methods

Preparation of lipid vesicles

Synthetic phospholipids, dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol

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(DMPG), dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) were purchased from Avanti Polar Lipis (Birmingham, AL, U.S.A.) and their homogeneity assayed by thin-layer chromatography [7]. The different lipid vesicles were prepared at 1 mg/ml phospholipid concentration in 30 mM Mops or Tris-HCl buffer (pH 7.0), containing 0.1 M NaCl, for 30 min in a water bath sonifier [5,8]. The temperature was maintained above the phase transition temperature value of the corresponding phospholipid. The main part of the vesicles prepared under these conditions is present as unilamellar vesicles based on the results obtained from freeze-fracture electron microscopy studies [5]. Lysophospholipids were not present in the vesicles as deduced from the chromatographic analysis of the lipid component.

Preparation of protein-lipid complexes

α-Sarcin was purified from cultures of *Aspergillus giganteus* MDH 18894. The isolation procedure was based on that described by Olson et al. [1]. The purified protein sample contains a small proportion of dimer, which is observed in polyacrylamide gels even under reducing conditions. This fact has been reported by Sacco et al. [9], who detected dimeric forms of α-sarcin in the monomer fraction isolated by gel filtration. This was confirmed through their sequence studies [9]. Nevertheless, the proportion of dimer in α-sarcin samples is lower than 10%. The amino acid composition and the spectroscopical properties of purified α-sarcin were coincidents to that previously described [10,11]. Lipid vesicles-protein complexes were obtained by adding α-sarcin to recently prepared vesicles at different lipid/protein molar ratios [4]. The protein concentration was determined by absorbance measurements at 280 nm based on the reported absorption coefficient [12].

Fluorescence depolarization measurements

Fluorescence measurements were performed on a Perkin Elmer MPF 44E spectrofluorimeter operated in the ratio mode. Cells of 0.2 cm optical path were used. The slit widths were 7 and 5 nm for the excitation and emission beams, respectively. Labelling of the phospholipid vesicles with 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-(trimethylamino)phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) was performed as previously described [13]. Emission of DPH and TMA-DPH was measured at 425 nm for excitation at 365 nm. The polarization of the fluorescence emission was measured in thermostated cells. The contribution of the protein to the polarization value of the fluorophores was negligible as deduced from the results of independent experiments. Succesive dilution for each sample was performed in order to check the potential contribution of the sample turbidity to the corresponding polarization degree. This was negligible at the protein/lipid molar ratios and optical paths used through these experiments.

Differential scanning calorimetry (DSC)

DSC analysis of the protein-lipid complexes was performed on a Microcal MC 2 at 30 C°/h speed, as previously described [8]. The calorimetric unit was interfaced to an IBM PC microcomputer for automatic data collection and analysis. Two different analyses were performed, with lipid-protein complexes prepared below and above the phase transition of the corresponding phospholipid. In both cases, a heating/cooling cycle was first completed. No differences on the further recorded scans were observed after repeated heating/cooling cycles.

Photolabelling

Labelling of α-sarcin with the lipid probes 1-palmitoyl-2-(2-azido-4-nitrobenzoyl)-*sn*-glycero-3-[^{3}H]phosphocholine (PC-I) and 1-myristoyl-2-(12-((4-azido-2-nitrophenyl)amino)dodecanoyl)-*sn*-glycero-3-[^{14}C]phosphocholine (PC-II) was performed in 30 mM Tris-HCl buffer (pH 7.0), containing 0.1 M NaCl as described [14]. Phosphatidylglycerol and phosphatidylcholine probe-containing vesicles were used for these studies. The experiments were performed at different lipid/probe molar ratios (from 100 to 7300 and from 264 to 19300 for PC-I and PC-II, respectively); the total radioactivity was kept constant at $5 \cdot 10^5$ dpm and $1 \cdot 10^5$ dpm for PC-I and PC-II, respectively. Radioactive vesicles were prepared at 1 mg/ml total lipid concentration. Sodium dodecylsulfate polyacrylamide gel electrophoresis of the samples, Coomassie blue staining, densitometric scanning of the gels, gel slicing and radioactivity counting were performed as described [14].

Results

Fluorescence depolarization studies

It has been reported that α-sarcin specifically interacts with acid phospholipid vesicles promoting their aggregation and fusion [4,5]. This specific interaction is also deduced when the influence of the protein on the thermotropic behaviour of different phospholipids bilayers is studied. The protein does not modify, at any extent, the phase transition of the zwitterionic phospholipid phosphatidylcholine, based on fluorescence anisotropy measurements of DPH-labelled DMPC and DPPC vesicles (data not shown). However, the thermotropic behaviour of acid phospholipid vesicles is clearly modified.

The steady-state anisotropy variation of DPH-labelled DMPG vesicles has been studied in the presence of different α-sarcin concentrations. A summary

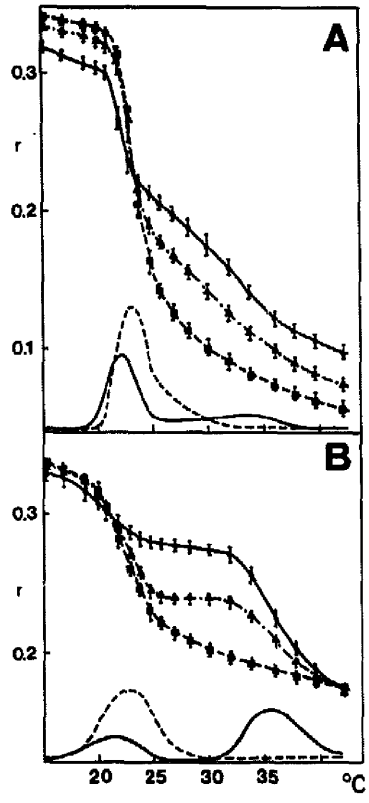

Fig. 1. Steady-state anisotropy (r) variation of (A) DPH- and (B) TMA-DPH-labelled DMPG vesicles versus temperature. The phase transition profiles correspond to different lipid/α-sarcin molar ratios: (●) 50:1, (▲) 125:1 and (■) DMPG alone. The studies were performed for samples in 30 mM Tris-HCl buffer (pH 7.0) containing 0.10 M NaCl. The phospholipid concentration was 80 μg/ml. The fluorescence probes were present at (DPH) 1:1000 and (TMA-DPH) 1:100 probe/lipid weight ratio. Anisotropy values were determined after equilibration of the samples for 10 min at each temperature. Values are expressed as the averages ± S.D. of three different determinations. The smooth curves resulting from the first-derivative analysis of the phase transitions corresponding to (– – –) DMPG and (———) DMPG/α-sarcin 50:1 molar ratio are also given; they are espressed in arbitrary units but the ones corresponding to the profiles in part B are plotted in a twice extended scale. Calculations were performed on a IBM PC-computer.

of the results obtained is given in Fig. 1A. The protein modifies the melting profile of the fluorescence labelled vesicles. All the observed effects become saturated at about 50:1 phospholipid/protein molar ratio. The transition temperature of the pure phospholipid (23°C) is not largely modified by α-sarcin, although an apparent decrease of about 0.5 C° is observed for this parameter at the saturating lipid/protein molar ratio. The amplitude of the anisotropy variation due to the phase transition of DMPG is decreased by the presence of α-sarcin. The effect of the protein is different below and above the melting temperature of the phospholipid. α-Sarcin decreases DPH polarization degree in the gel phase of the lipid bilayer and increases the value of this parameter in the liquid-crystalline phase. The maximum decrease observed below the phase transition is about 0.02 steady-state anisotropy units, and this parameter increases about 0.04 units above the melting temperature. Thus, α-sarcin would produce a decrease on the order of the lipid molecules below the phase transition temperature and a concomitant ordering on the liquid-crystalline state.

It is also remarkable the apparent presence of a broad transition mainly observed at high protein/lipid molar ratios. This non-well defined transition, which would be centered at about 37°C, is suggested from the first-derivative analysis of the melting profile corresponding to α-sarcin/DMPG at the saturating lipid/protein molar ratio (Fig. 1A).

All the described effects are not observed at 0.5 M ionic strength (data not shown), in agreement to the reported decreased α-sarcin–lipid interaction under such conditions [4].

It is well accepted that DPH occupies the hydrophobic core region of lipid bilayers [15], whereas its synthetic derivative TMA-DPH is anchored to the lipid/water interface through its non-fluorescent moiety [16]. Thus, TMA-DPH would give information concerning to a more defined region than DPH. In order to assess the effect of α-sarcin near the polar headgroup regions of the bilayers, the anisotropy variation of TMA-DPH-labelled DMPG vesicles has been also analyzed. The obtained results are summarized in Fig. 1B. The phase transition of DMPG vesicles monitored by measuring the fluorescence polarization of TMA-DPH is broader than the observed for DPH-labelled vesicles. The widths at the half-height of the corresponding first-derivative peaks are 4 and 5.5 C° for DPH- and TMA-DPH-labelled vesicles, respectively. Nevertheless, the melting temperature of the pure lipid is coincident for both probes.

Two well defined transitions are observed in this case. The first transition appears at about 22°C and the second one is centered at about 35°C. The relative contribution of that appearing at high temperature increases as the protein/lipid molar ratio rises (Fig. 1B). This last one may be related to the non-well defined transition suggested from the melting profile of DPH-labelled vesicles. This transition would represent a stabilization of the bilayer at the polar headgroup region, since TMA-DPH essentially probes the glycerol backbone region and the fatty acid acyl chain regions near the polar head of the phospholipid. This stabilization would be very significant since is accompanied by a displacement of more than 10 C°, from 22 to 35°C, on the midpoint of the transition monitored by fluorescence depolarization measurements. A similar large displacement of T_m has been also reported for DPH-

DPPG vesicles in the presence of poly(Lys,Tyr), also by measuring DPH fluorescence depolarization [17].

The biphasic behaviour observed would indicate the existence of different lipid domains of significant size. Considering the location of the two fluorescence probes in the bilayer, these results would reflect the existence of a surface interaction between α-sarcin and phosphatidylglycerol vesicles although also affecting the inner lipid core.

The results obtained for the interaction between α-sarcin and DPPG for both types of fluorescence probe-labelled vesicles are given in Fig. 2. They are similar to those corresponding to the α-sarcin–DMPG interaction. The lipid-protein system also becomes saturated at 50:1 phospholipid/protein molar ratio and two transitions are also detected. The relative amplitude of the one appearing at high temperature is lower for DPPG than for DMPG. According to these results, the surface interaction of α-sarcin with phosphatidylglycerol vesicles would be produced in a lower extent

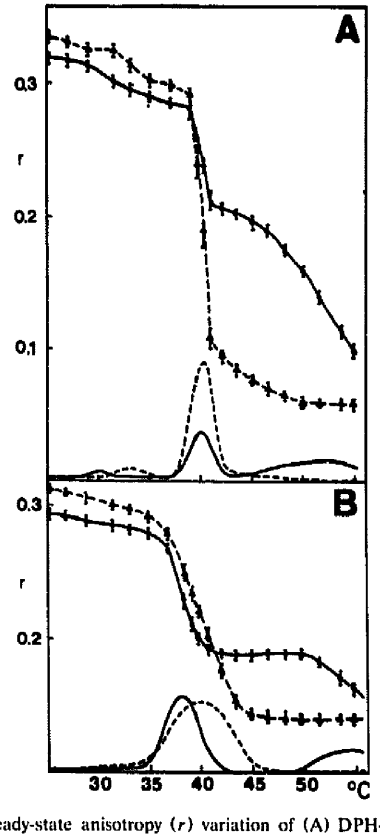

Fig. 2. Steady-state anisotropy (r) variation of (A) DPH- and (B) TMA-DPH-labelled DPPG vesicles versus temperature, in the absence (▲) and in the presence (●) of α-sarcin at 50:1 lipid/protein molar ratio. First-derivative curves of the two melting profiles are also included. Other experimental details as in the legend of Fig. 1.

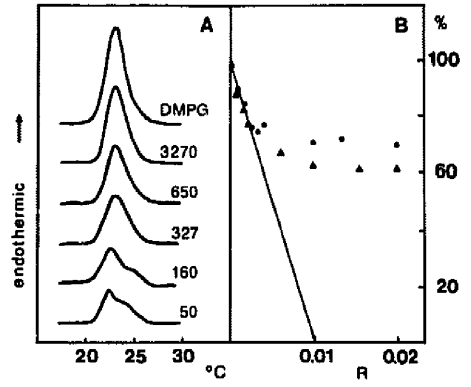

Fig. 3. (A) Differential scanning calorimetric thermograms for DMPG vesicles, at different lipid/α-sarcin molar ratios (ratios are expressed per unit of protein), prepared at temperature above the T_m of the phospholipid. No enthalpy variation is observed above 30°C (from 30 to 40°C) in these thermograms. A heating/cooling cycle was first completed. The scans were obtained under reheating conditions (see Methods). No differences were observed after repeated heating/cooling cyles. (B) Relative enthalpy variation (%) versus α-sarcin/DMPG molar ratio (R) for protein-vesicles complexes prepared (▲) above and (●) below the T_m of the phospholipid. Values are referred to the enthalpy variation associated to the thermal transition of DMPG alone, and were calculated as the area under the whole calorimetric scan. The straight line corresponds to the extrapolation to zero of the enthalpy variation.

as the length of the fatty acid acyl chain increases. Differences have also been described for the thermal behaviour of DPPG and DMPG vesicles upon interaction with myelin basic protein [18].

Differential scanning calorimetry

The effect of α-sarcin on the thermotropic behaviour of phospholipid vesicles was also analyzed by DSC. The protein does not modify the calorimetric profile of phosphatidylcholine vesicles in agreement with the fluorescence depolarization measurements. However, changes are detected for phosphatidylglycerol vesicles upon addition of α-sarcin. The results obtained are given in Fig. 3. Increase of the α-sarcin concentration produces a broadening of the calorimetric peak, although the position of its maximum remains almost unchanged. A shoulder is observed in the calorimetric scan of samples for protein concentrations higher than 1:160 α-sarcin/DMPG molar ratio. This shoulder appears at about 24.5 °C and it cannot be attributed to the protein itself since α-sarcin shows an endothermic transition at about 50°C. The relative enthalpy variation associated to the thermal transition has been calculated as the area under the whole calorimetric scan. A plot of this magnitude versus protein concentration shows a saturating behaviour (Fig. 3B). The value of the enthalpy variation decreases from 7.8 kcal/mol for pure phospholipid to 4.8 kcal/mol for

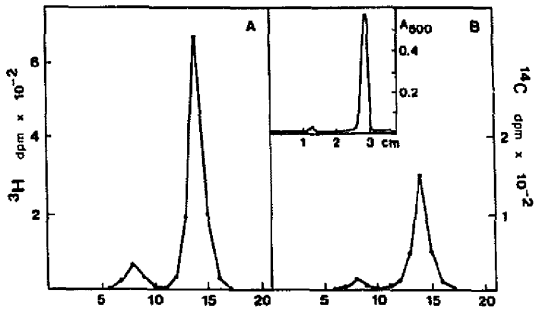

Fig. 4. Hydrophobic labelling of α-sarcin with photoactive DMPG vesicles. The results correspond to vesicles containing (A) PC I and (B) PC II probes, respectively. Radioactivity values are given for each 2-mm slice from the corresponding gel. Electrophoretic migration was from left (−) to right (+). The lipid/protein molar ratio used in the photolabelling experiments was 150:1. The minor peaks appearing in both panels correspond to the dimer fraction of α-sarcin (see Methods). Inset, densitometric scan of protein-stained gels.

DMPG/α-sarcin complex at 50:1 molar ratio. This represents about a 40% decrease. If the α-sarcin-DMPG complexes are prepared below the transition temperature of the phospholipid instead above this temperature, a similar saturating plot is observed for the enthalpy variation although about 30% decrease is observed for this magnitude in this case (Fig. 3B). The phase transition temperature is not modified upon addition of the protein. But the shoulder appearing at high temperature, observed when the protein-lipid complex are formed above the phase transition, is not detected even at the saturating protein concentration. A single gaussian band thermograms are observed in the temperature range considered (up to 40°C) for all the lipid/protein ratios studied.

Hydrophobic photolabelling

The results obtained from the hydrophobic photolabelling of α-sarcin in DMPG vesicles-protein complexes are given in Fig. 4. They were obtained after 1 h of incubation of the protein in the presence of the lipid vesicles. But a kinetic study revealed that 70% of the total labelling was incorporated to the protein after only 2 min. The usefulness of this methodology to analyze protein–lipid interactions has been already discussed [19,20]. Two photoactive probes PC I and PC II have been used in these experiments. PC I carries the photoactive group at level of the polar headgroup. Thus, it labels those regions of the protein exposed to lipids at surface level, whereas PC II is a probe for the lipid core of the bilayer. After preparation of the protein-vesicles complexes, the samples were irradiated with long wavelength ultraviolet light. The protein was extracted from the lipid-protein complex [19,20] and further analyzed by SDS polyacrylamide gel electrophoresis and the radioactivity along the gel was measured by counting of 2 mm slices. No photolabelling of α-sarcin was observed when either PC I or PC II were incorporated in phosphatidylcholine vesicles which agrees with the results obtained by fluorescence polarization and DSC measurements. But, two radioactive peaks are observed (Fig. 4) when either PC I- or PC II-containing DMPG vesicles were used. The amount of radioactivity incorporated to the protein is similar to that reported for the hydrophobic photolabelling of diphtheria toxin under the same experimental conditions [21]. These two peaks correspond to the monomer and dimer fractions present in α-sarcin samples (see Preparation of protein-lipid complexes under Methods). This is corroborated by the densitometric scans of the corresponding gels after protein staining (Fig. 4 inset). The results obtained show significant labelling of the protein with both the superficial probe PC I and the deeper one PC II. These results would indicate the existence of both surface and inner interaction of α-sarcin with DMPG vesicles.

Discussion

We have reported that α-sarcin interacts with negatively charged phospholipid vesicles. This interaction produces lipid-protein complexes which can be isolated by centrifugation in a sucrose gradient [4,5]. These complexes are formed by aggregated and fused vesicles. Previously reported results revealed the existence of a temperature-dependent transition for the process of formation of large size complexes when both fusion and aggregation assays were considered [4,5]. The midpoint of this transition was near to the T_m value of the phase transition of the corresponding phospholipid. According to both types of assays, aggregation and fusion, the effect of α-sarcin is higher at temperatures above the T_m of the lipid considered, although it is also observed at temperatures below the T_m value but in a lower extent. All these observations suggest a clear relationship between lipid state and α-sarcin–phospholipid interaction, which agrees with the results now reported.

α-Sarcin does not affect the thermotropic transition of phosphatidylcholine vesicles but clearly alters the behaviour of phosphatidylglycerol vesicles. According to the results obtained from measurement of the fluorescence depolarization of TMA-DPH-labelled DMPG vesicles, the α-sarcin–phospholipid interaction would exhibit an electrostatic component responsible for the observed upwards shift in the T_m value. Phosphatidylglycerol apparently does not participate in intermolecular hydrogen bonding and the charged groups of DMPG would repel each other [22]. It is also known that fully protonated phosphatidylglycerols present a main phase transition increased in temperature by approx. 17 C° [23]. Therefore, an electrostatic interaction between

the positive charges of α-sarcin and the negative groups of DMPG, would produce a repulsive charge neutralization and an increased T_m value related to stabilization of the bilayer. However, such an effect of α-sarcin is less evident when the phase transition of DMPG vesicles is monitored by DPH-labelling. This would indicate that the effect of α-sarcin on the lipid ordering is different at the surface (probed by TMA-DPH) than at the hydrophobic core (probed by DPH) of the bilayer.

The transition observed at high temperature for TMA-DPH labelled vesicles is not observed by DSC analysis of the α-sarcin-phospholipid complexes prepared either below or above the phase transition temperature of the phospholipid. The reasons for this apparent discrepancy are unclear but may be related to the local perturbations in the phosphatidylglycerol bilayer induced by the fluorescence polarization probe. Raman spectroscopic studies about poly(L-Lys)–DPPG vesicles interaction revealed an increase in intrachain molecular order in the gel as well as the liquid crystalline states [24]; however, fluorescence polarization measurements of DPH-DPPG vesicles revelaled that poly(L-Lys) decreases the degree of DPH fluorescence polarization in the gel phase of DPPG while increasing fluorescence polarization in the liquid crystalline phase [25]. This last is in agreement to the results obtained from electron spin resonance spectroscopic studies for the same system [26]. These different results were potentially related to the local perturbations induced by the spectroscopic probes in the bilayer [27]. The effect of the G protein, from the vesicular stomatitis virus, on the DPPC gel to liquid crystalline phase transition has been studied by using different fluorescence probes [28,29]. These probes could detect a DPPC phase transition at G protein concentrations well above that at which the calorimetric results [30] predict a complete abolition of the DPPC chain-melting transition. A potential explanation for this apparent discrepancy may be related to a different nature of the thermotropic transitions monitored by DSC and fluorescence spectroscopy which may be not completely comparable [27]. This may occur for the α-sarcin–phosphatidylglycerol interaction. In this sense, it is noteworthy that TMA-DPH and DPH also render different melting profiles.

Calorimetric analysis of the α-sarcin-DMPG complexes shows a decrease in the enthalpy variation asociated to the lipid phase transition as the protein molar ratio increases in the sample. Such a plot exhibits a saturating behaviour. The break in the linear decrease of the enthalpy variation appears at about 200:1 DMPG/α-sarcin molar ratio. Extrapolation to zero enthalpy variation (Fig. 3B) renders a value of about 100 ± 25 phospholipid molecules affected by each α-sarcin molecule. This value may be related to the number of molecules that the protein could have removed from participation in the phase transition. However, this is a high value for a small and no hydrophobic protein no matter how it is disposed in the bilayer. Thus, it is rather related to extensive hydrophilic lipid–protein interactions which reduce the energy of the acyl chain melting transition. This pattern was also observed for the interaction of cytohcrome *P*450 and DMPC and was interpreted in terms of saturation of liposomes with the protein and a predominant adsorption of the excess protein on vesicles surfaces due to hydrophilic interactions only [31].

The shoulder at 24.5°C observed in the calorimetric scans of protein-lipid complexes, when these are prepared above the T_m value of the phospholipid and at high α-sarcin concentration, could be related to the presence of large structures among such complexes. A similar shoulder has been also observed when analyzing the influence of the curvature of lipid model membranes on their phase transition [32] and these authors also considered such a possibility to explain its presence. The absence of the above mentioned shoulder in the calorimetric scans of α-sarcin-DMPG complexes prepared below the phase transition of the lipid would be in agreement with that posibility. In fact, when A_{360} versus temperature was measured for α-sarcin-DMPG the values obtained above the T_m were higher than that observed below the phase transition of the phospholipid [4]. Since the A_{360} would be related to the size of the particles responsibles for the light-scattering measured, the size of the lipid complexes would be larger above than below the T_m of the lipid. Moreover, the absorbance variation is not linear, showing a deep break at about the T_m value of the lipid considered, indicating that the differences on the size of the complexes above and below the T_m should be very significants. But, the α-sarcin-DMPG complexes are not interconverted appearing different structures if the protein-lipid complex is prepared above or below the T_m of the phospholipid. Repeated heating DSC scans of α-sarcin-phospholipid complexes prepared below the transition temperature do not show the shoulder at 24.5°C exhibited by the samples prepared above the T_m. This may be related to the existence of non-reversible metastable states for the α-sarcin-phospholipid vesicles. A metastable state appearing in a not reversible process has been proposed for the myelin basic protein–DMPG interaction to explain the different thermograms obtained depending on the temperature of preparation of the samples [22]. This behaviour may be due to different hydrophobic interaction of the protein with the bilayer above and below the T_m of the phospholipid.

The analyses performed by hydrophobic photolabelling of α-sarcin in DMPG-protein complexes indicate the existence of both surface and inner lipid–pro-

tein interaction. Moreover, they reveal that α-sarcin penetrates the bilayer deep enough to be labelled with the inner probe. This penetration of α-sarcin in the bilayer should not be surprising. In fact, there is considerable evidence indicating that even the binding of poly(L-lysine) and lysine copolymers to anionic phospholipids should not be considered as a simple electrostatic interaction (see Ref. 27 for a review). Moreover, it has been reported that α-sarcin is partially protected from trypsin hydrolysis when interacting with lipid vesicles [4,33] which also suggests penetration of the protein into the bilayer.

α-Sarcin, a water-soluble and hydrophilic protein, would interact with phospholipid bilayers by a combination of electrostatic and hydrophobic forces. The protein would initially adsorb to the charged polar head group of the phospholipids and further would partially penetrate the hydrophobic/hydrophilic interface of the bilayer to interact with a portion of the lipid hydrocarbon chains. This is a characteristic behaviour of type 2 proteins (according to the systematic surveys of lipid–protein interactions, Refs. 27 and 34) which also normally increase the permeability of phospholipid vesicles. In this sense, α-sarcin produces fusion of phosphatidylglycerol vesicles with concomitant alteration of the bilayer permeability which results in calcein release from vesicles containing trapped calcein [5].

The obtained results reveal that α-sarcin displays many common features with other proteins also able to interact with phosphatidylglycerol vesicles, as the myelin basic protein [18,26,34,35] or the matrix protein from the vesicular stomatitis virus [36–39]. These two proteins are basic polypeptides lacking of long stretches of hydrophobic amino acids in their sequences. However, both of them interacts with negatively charged lipid vesicles through hydrophobic and electrostatic forces. α-Sarcin is also a basic protein, of a similar size to myelin basic protein, and also lacks of long enough segments to be considered as candidates for hydrophobic interaction with lipids. However, there are not sequence homologies between α-sarcin and these proteins which could explain the similar ability to interact with lipid vesicles. Only there is a common feature between myelin basic protein and α-sarcin. The former protein contains a triproline segment, located at the middle of the molecule, which would sharply bend the polypeptyde. α-Sarcin has a disulfide bond which brings close the NH_2- and COOH-terminal ends of the molecule, also bending the polypeptide chain. This structural pattern and the basic character of α-sarcin may be the reasons for its particular ability to interact with phosphatidylglycerol vesicles. This interaction presumably is involved in the pass of the protein across cell membranes and consequently in the antitumour character of α-sarcin.

Acknowledgments

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