

Food mustard allergen interaction with phospholipid vesicles

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Sin a I, the major allergen from mustard seeds, interacts with acid phospholipid vesicles. The protein binds to dimyristoylglycerophosphoglycerol vesicles with an apparent dissociation constant of approximately 2.4 μ M, the number of phospholipid molecules affected by one protein molecule being approximately 20. *Sin a* I promotes an increase in the light scattering of a vesicle suspension. This process becomes saturated at approximately a lipid/protein molar ratio of 20:1. *Sin a* I also modifies the thermotropic behaviour of the negatively charged vesicles, which has been studied by measuring the fluorescence polarization of the probe 1,6-diphenyl-1,3,5-hexatriene incorporated into the hydrophobic core of the bilayer. *Sin a* I also promotes lipid mixing between vesicles. This mixing has been analyzed by measuring the variation of the fluorescence energy transfer between *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-dimyristoylglycerophosphoethanolamine (donor) and *N*-(lissamine rhodamine B sulphonyl)-PtdEtn (acceptor) incorporated into dimyristoylglycerophosphoglycerol vesicles. This effect is also corroborated by observing a single thermotropic transition in a mixture of independent dipalmitoylglycerophosphoglycerol and dimyristoylglycerophosphoglycerol vesicles when *Sin a* I is added to the lipid suspension. The allergen promotes release of aqueous contents of PtdGro vesicles, as determined by an aminonaphthalenetrisulfonic acid/*p*-xylylenebis-(pyridinium)bromide dequenching assay. This study shows that the allergen *Sin a* I is able to interact with membrane lipids. This interaction is discussed in terms of its potential involvement in the allergenicity of this protein.

Atopic allergy diseases (type-I allergies) are common clinical disorders in developed countries, food being one of the most relevant biological sources of type-I allergens [1]. Many seed spices have been shown to be allergenic, e.g. table mustard, which is the most significant in terms of the number of individuals affected [2, 3]. The major allergen from table mustard is *Sin a* I [4]. It is a small and basic protein (14.1 kDa) isolated from the seeds of *Sinapis alba* (yellow mustard) [5], and composed of two different polypeptide chains (39 and 88 amino acid residues) linked by disulphide bridges [4]. This protein is a typical storage 2S albumin, which is believed to be involved in providing nitrogen and sulfur to the germinating seedling.

The overall requirements that a molecule should accomplish to cause allergic reactions are unknown, but the existence of common structural or biochemical properties shared by type-I allergens is conceivable although this has not been found so far. Allergens increase the levels of their specific IgE antibodies and trigger the release of inflammatory medi-

ators from mast cells and basophils. Therefore, the access of allergenic molecules into the systemic circulation should be inherent to the type-I allergies. When considering food allergens such as *Sin a* I, such an access would require the passage of the protein through the gut epithelial barrier since otherwise the modulation of the specific IgE synthesis at the T-cells and B-cells would be difficult. Thus, allergenicity may be related to the ability of a polypeptide molecule to cross membranes, which may not be related to its natural role. In this sense, the binding of melittin, an allergen from bee venom, to cell membranes has been demonstrated to be correlated with an enhancement of its immunogenicity [6]. Based on these considerations, some kind of interaction between type-I allergens and membranes may be expected. We have considered this possibility for *Sin a* I. This study reveals a strong interaction between the major mustard allergen *Sin a* I and lipid bilayers. We describe the results about this interaction, and its potential biological implications are discussed.

MATERIALS AND METHODS

Purification of the allergen *Sin a* I

Sin a I was isolated from mustard *S. alba* L. flour seeds by saline extraction, followed by gel filtration using Sephadex G-50 and finally ion-exchange chromatography with SP Sephadex C-25 [5]. The concentration of the purified protein

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Abbreviations. Myr₂GroPCho, dimyristoylglycerophosphocholine; Myr₂GroPEtn, dimyristoylglycerophosphoethanolamine; Myr₂-GroPGro, dimyristoylglycerophosphoglycerol; Ole₂GroPGro, dioleoylglycerophosphoglycerol; Pam₂GroPGro, dipalmitoylglycerophosphoglycerol.

solutions was determined from absorbance measurements at 280 nm using an absorption coefficient [0.1% (mass/vol.), 1-cm light path] of $0.511 \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ [4]. Protein samples isolated according to this procedure are of sufficient purity for sequence analysis of the allergen [4].

Preparation of the lipid vesicles

Synthetic phospholipids, dimyristoylglycerophosphoglycerol, dipalmitoylglycerophosphoglycerol and dioleoylglycerophosphoglycerol (Myr₂GroPGro, Pam₂GroPGro and Ole₂GroPGro, respectively), dimyristoylglycerophosphocholine (Myr₂GroPCho), *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-dimyristoylglycerophosphoethanolamine (Myr₂GroPEtn) and *N*-(lissamine rhodamine B sulphonyl)-PtdEtn were purchased from Avanti Polar Lipids. Vesicles composed of these lipids were prepared at 1 mg/ml phospholipid in 50 mM Mops, pH 7.0, containing 0.1 M NaCl, by sonication for 30 min in a Branson water-bath sonifier [7]. The temperature was maintained 5°C above the phase transition value of the corresponding phospholipid. Aliquots of the phospholipid vesicles were assayed for lipid concentration [8]. Lysophospholipids were not detected by chromatographic analysis of the lipid component after the preparation of the vesicles [9]. The average size of these vesicles is approximately 100 nm as deduced from electron micrographs [10]. Lipid-protein complexes were obtained by adding *Sin a I* to recently prepared vesicles at the required lipid/protein molar ratio.

Binding of the allergen to the lipid vesicles

The protein-vesicle binding experiments were performed by incubation of the phospholipid vesicles with increasing amounts of *Sin a I* in 50 mM Mops, pH 7.0, containing 0.1 M NaCl, for 30 min at 37°C. Protein-binding measurements were performed from two sets of experiments. The free, unbound protein, was separated by ultrafiltration on Millipore membranes [7] or centrifugation of the protein-lipid mixtures. These were centrifuged for 40 min at $130000 \times g$ on an Airfuge (Beckman, rotor A95). The concentration of unbound protein was determined by absorbance measurements of the corresponding supernatants, using the above-mentioned absorption coefficient. Control experiments with radioactive phospholipid vesicles were performed; in our experimental conditions the contribution of the free vesicles to the absorbance was negligible in the absence of protein. The analysis of the binding data was carried out as described previously [7].

Aggregation of the vesicles

The aggregation of the lipid vesicles induced by *Sin a I* was studied by continuously measuring the absorbance variation produced by addition of the protein to recently prepared lipid vesicles. The light scattered by the aggregated vesicles produces an apparent absorbance in the visible spectral region. Absorbance measurements at 360 nm were performed on a Beckman DU-8 spectrophotometer equipped with a thermostated cell holder. Cells of 1-cm optical path length were used. Control samples in the absence of the protein were considered for each experiment.

Lipid mixing assays

Lipid mixing assays of membrane phospholipids induced by *Sin a I* was measured by energy-transfer assays [11]. The

extent of the lipid mixing was estimated from the increase of the *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-Myr₂GroPEtn fluorescence (emission band centered at 530 nm). Typically, a vesicle population containing 1% molar ratio of each fluorescent lipid, *N*-(lissamine rhodamine B sulphonyl)-PtdEtn (acceptor) and *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-Myr₂GroPEtn (donor), was mixed with unlabelled phospholipid vesicles at a 1:9 molar ratio. Emission spectra for excitation at 450 nm were recorded on a SLM Aminco 8000 spectrofluorimeter after a 40-min incubation at 37°C. The efficiency of the energy transfer is defined by the equation $ET (\%) = (1 - F/F_0) \times 100$ where F and F_0 are the fluorescence emission intensities at 530 nm in the presence and absence of *N*-(lissamine rhodamine B sulphonyl)-PtdEtn, respectively, and ET is the energy transfer [11]. The fluorescence emission values were normalized for the donor/acceptor concentrations present in each particular sample by disrupting the vesicles with 1% Triton X-100 [10].

Fluorescence depolarization measurements

These measurements were performed on a SLM Aminco 8000 spectrofluorimeter equipped with 10 mm Glan-Thompson polarizers. Cells of 0.2-cm optical path length were used. The slit widths were 4 nm for both the excitation and emission beams. Labelling of the phospholipid vesicles with 1,6-diphenyl-1,3,5-hexatriene was performed as previously described [12]. Emission of the fluorescence probe was measured at 425 nm for excitation at 365 nm. The contribution of sample turbidity to the polarization of the fluorescence emission was negligible for the concentrations and optical paths used. This was verified by measuring the degree of polarization of successive dilutions for each sample. The steady-state polarization of the fluorescence emission was measured in thermostated cells. The temperature of the sample was determined by using an inner temperature probe.

Release of vesicle aqueous contents

This analysis was performed with vesicles containing both 8-aminonaphthalene-1,3,6-trisulfonic acid and *p*-xylylenebis(pyridinium)bromide (Molecular Probes). *p*-Xylylenebis(pyridinium)bromide is highly water-soluble and efficiently quenches 8-aminonaphthalene-1,3,6-trisulfonic acid fluorescence when both are encapsulated together in the same vesicles. Release of aqueous contents prevents the quenching as a consequence of the dilution of both 8-aminonaphthalene-1,3,6-trisulfonic acid and *p*-xylylenebis(pyridinium)bromide into the external medium [13]. Egg PtdGro (Sigma) vesicles containing both 8-aminonaphthalene-1,3,6-trisulfonic acid and *p*-xylylenebis(pyridinium)bromide were prepared (at 2 mg/ml phospholipid concentration) by five cycles of freeze thawing and further extrusion through polycarbonate membranes (0.1-μm pore diameter, Nuclepore) in an extruder (Lipex Biomembranes). Vesicles were prepared in 10 mM Tris, pH 7.4, containing 20 mM NaCl, 12.5 mM 8-aminonaphthalene-1,3,6-trisulfonic acid and 45 mM *p*-xylylenebis(pyridinium)bromide. Non-encapsulated material was removed by gel filtration on a Sephadex G-75 column, equilibrated in 10 mM Tris, pH 7.4, containing 100 mM NaCl and 1 mM EDTA [14]. The fluorescence emission of 8-aminonaphthalene-1,3,6-trisulfonic acid was measured through a Corning 3-68 cut-off filter (greater than 530 nm) for excitation at 386 nm, on a SLM Aminco 8000 spectrofluorimeter. The fluorescence emission of the vesicles

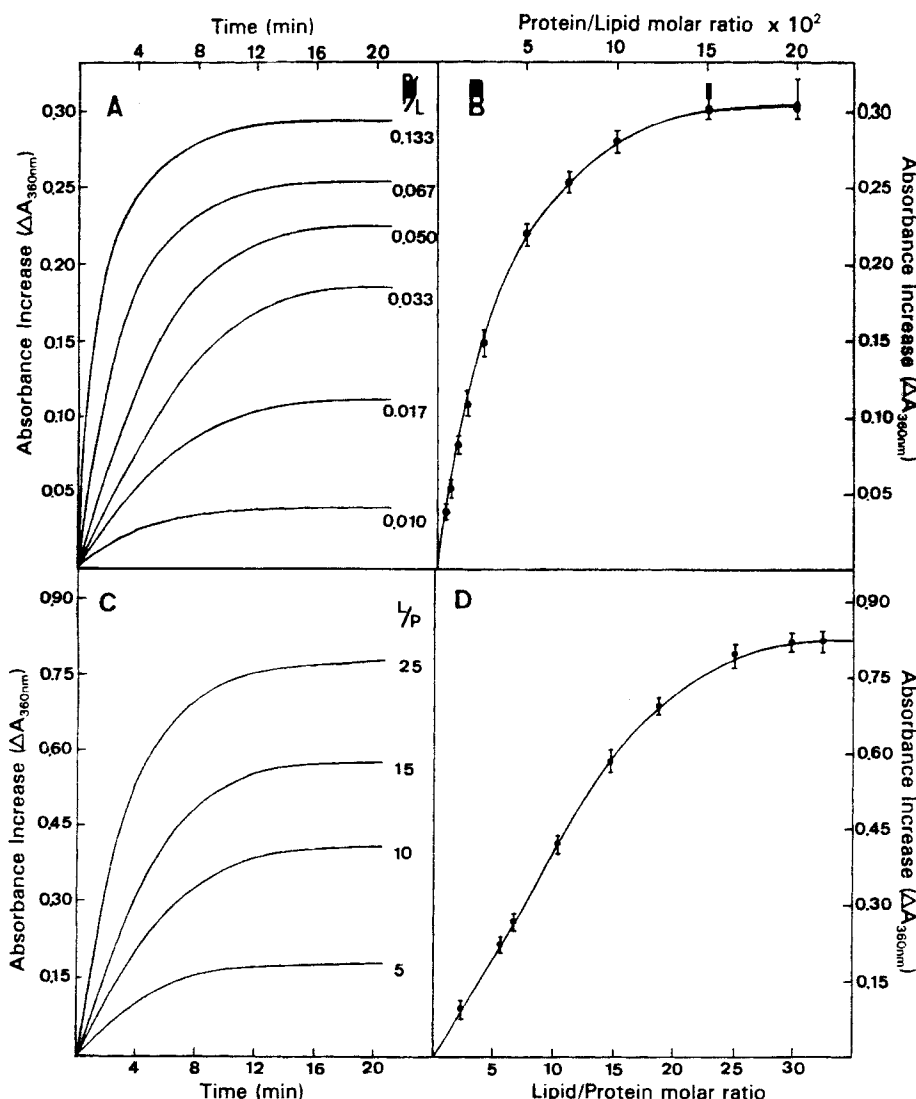


Fig. 1. Variation of the absorbance at 360 nm resulting from the interaction between *Sin a I* and Myr₂GroPGro vesicles. (A, C) Time course of the variation induced at 37°C. Saturating absorbance variation values versus (B) protein/lipid and (D) lipid/protein molar ratios. (A, B) 30 μM Myr₂GroPGro in 50 mM Mops pH 7.0, containing 0.1 M NaCl, was titrated by adding *Sin a I*. (C, D) 4.7 μM *Sin a I* in the same buffer was titrated with Myr₂GroPGro vesicles. Cells of 1-cm optical path length were used for all the measurements. Averages $\pm$ SD of three different experiments are given.

containing both 8-aminonaphthalene-1,3,6-trisulfonic acid and *p*-xylylenebis(pyridinium)bromide was set to 0% leakage, while the fluorescence emission of the lysed vesicles (by adding 1% Triton X-100) was set to 100% leakage. Results are expressed relative to this value.

RESULTS

Aggregation of phospholipid vesicles induced by *Sin a I*

The interaction of *Sin a I* with phospholipid vesicles is easily detected by observing the resulting turbidity upon addition of the protein to a vesicle suspension. This optical effect results from the scattered light by the enlarged particles produced by the allergen-vesicle interaction. The time course of this process was analyzed by measuring the absorbance at 360 nm. A summary of the results obtained with Myr₂GroPGro vesicles is given in Fig. 1A, C. This kinetic study reveals that the absorbance variation reaches equilibrium after different periods of time depending on both phospholipid and

protein concentrations. Nevertheless, the main absorbance increase occurs within the first few minutes, although approximately 20 min are required for completion of the process at the lowest protein/lipid molar ratio tested. A plot of the maximum absorbance variation versus *Sin a I*/Myr₂GroPGro molar ratio is given in Fig. 1B, when phospholipid vesicles are titrated by adding protein. The observed saturating protein phospholipid molar ratio is 1:22. A similar result is also observed for the titration of *Sin a I* with Myr₂GroPGro vesicles, and the same saturating lipid/protein molar ratio is obtained (Fig. 1D).

This effect produced by the allergen *Sin a I* does not seem to be dependent on either the length or the degree of unsaturation of the acyl chains of the phospholipid, since similar results have been obtained for Pam₂GroPGro, Ole₂-GroPGro and egg PtdGro vesicles (data not shown). These experiments have also been performed with the zwitterionic phospholipid Myr₂GroPCho, and no absorbance variation has been detected throughout the considered protein concentration range.

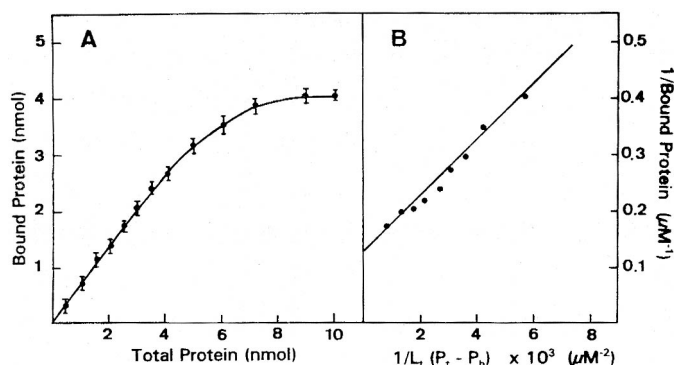


Fig. 2. Binding of *Sin a I* to Myr₂GroPGro vesicles. (A) Bound protein versus total protein added. The studies were performed in 50 mM Mops, pH 7.0, containing 0.1 M NaCl. The phospholipid concentration employed was 167 μ M. Results are given as averages $\pm$ SD of three independent experiments. (B) P_b , bound protein; P_t , total protein; L_t , total phospholipid concentrations (see Results section).

Analysis of the binding of *Sin a I* to phospholipid vesicles

The protein binds to the phospholipid vesicles. The binding results have been analysed by considering

$$(P) \text{ protein} + \text{free lipid sites} = PL_n,$$

as the interaction equilibrium. Thus, a dissociation constant, K_d , can be defined as a function of (P_t) total protein, (P_b) bound protein, (L_t) total phospholipids and n , the number of phospholipid molecules that would provide one binding site for the protein molecule P . The following equation can be deduced [6]:

$$1/P_b = [K_d n/L_t (P_t - P_b)] + n/L_t.$$

A plot of $1/P_b$ versus $1/L_t(P_t - P_b)$ allows the calculation of both K_d and n . A summary of the obtained results for Myr₂GroPGro vesicles is given in Fig. 2. The calculated binding parameters are 22 and 2.36 μ M for n and K , respectively. Thus, the number of phospholipid molecules affected by a protein molecule is approximately 20. In protein-lipid interaction, the notion of binding site must be related to the number of phospholipid molecules affected by the protein, due to the lateral diffusion of the phospholipid. The obtained n value is in good agreement with the saturating stoichiometry deduced from the aggregation results.

Effect of *Sin a I* on the thermotropic behaviour of the phospholipids

The interaction between acid phospholipid vesicles and *Sin a I* is also deduced by examining the influence of the protein on the thermotropic behaviour of different phospholipid bilayers. These studies have been performed by using fluorescence depolarization measurements. The thermotropic behaviour of the acid phospholipid Myr₂GroPGro vesicles is clearly modified (Fig. 3). The steady-state anisotropy variation of Myr₂GroPGro vesicles labelled with 1,6-diphenyl-1,3,5-hexatriene has been studied in the presence of different phospholipid *Sin a I* molar ratios. The protein progressively decreases the anisotropy of the fluorescence probe in the gel phase of the lipid bilayer while it increases the value of this parameter in the liquid-crystalline phase. *Sin a I* would produce a decrease in the order of the lipid molecules below the phase transition temperature and a concomitant ordering in

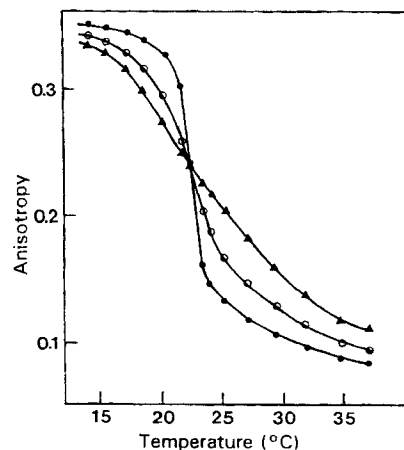


Fig. 3. Thermal variation of the steady-state anisotropy of Myr₂GroPGro vesicles labelled with 1,6-diphenyl-1,3,5-hexatriene. The fluorescence probe was present at a 1:1000 probe/lipid mass ratio. The phase transition profiles correspond to different lipid/*Sin a I* molar ratios: Myr₂GroPGro alone ($\bullet$); 50:1 ($\circ$); 25:1 ($\blacktriangle$). The phospholipid concentration was 80 μ g/ml and the studies were performed in 50 mM Mops, pH 7.0, containing 0.1 M NaCl. Anisotropy was determined after equilibration of the sample for 10 min at each temperature. Values are expressed as the average of three different determinations.

the liquid-crystalline state. Thus, the protein decreases the amplitude of the gel to liquid-crystalline thermal transition of Myr₂GroPGro bilayers and tends to abolish their cooperative character. However, the transition temperature of the phospholipid is not modified. The observed effects become saturated to a phospholipid/protein molar ratio of approximately 25:1. The allergen *Sin a I* does not modify, to any extent, the phase transition of the zwitterionic phospholipid Myr₂GroPCho, based on fluorescence anisotropy measurements of labelled vesicles (data not shown). This result may be expected since no aggregation has been observed upon addition of the protein to these vesicles.

Lipid mixing produced by *Sin a I*

The allergen causes lipid mixing of bilayers of acid vesicles at pH 7. This has been demonstrated by measuring the decrease in the efficiency of the fluorescence energy transfer between *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-Myr₂GroPEtn (donor) and *N*-(lissamine rhodamine B sulphonyl)-PtdEtn (acceptor) incorporated into Myr₂GroPGro vesicles. Fig. 4A shows the emission spectrum (b) for excitation at 450 nm of vesicles containing both donor and acceptor probes and unlabelled vesicles (1:9 molar ratio) in the absence of protein. Two emission maxima at wavelengths corresponding to donor (530 nm) and acceptor (590 nm) are observed. Addition of *Sin a I* to a mixture of both fluorescence-labelled and unlabelled Myr₂GroPGro vesicles (1:9 molar ratio) caused an increase in the intensity of the emission peak of the donor (530 nm) and a concomitant reduction in the emission peak corresponding to the acceptor (590 nm). These changes indicate a reduction in the efficiency of the donor-to-acceptor resonance energy transfer in the presence of the protein. The fluorescence emission variations occur in a very short period of time; the main increase in fluorescence intensity appears within a few seconds. Nevertheless, in order to consider only the energy transfer variation of the final phospholipid/*Sin a I* complexes formed, the spectra shown in Fig. 4 have been

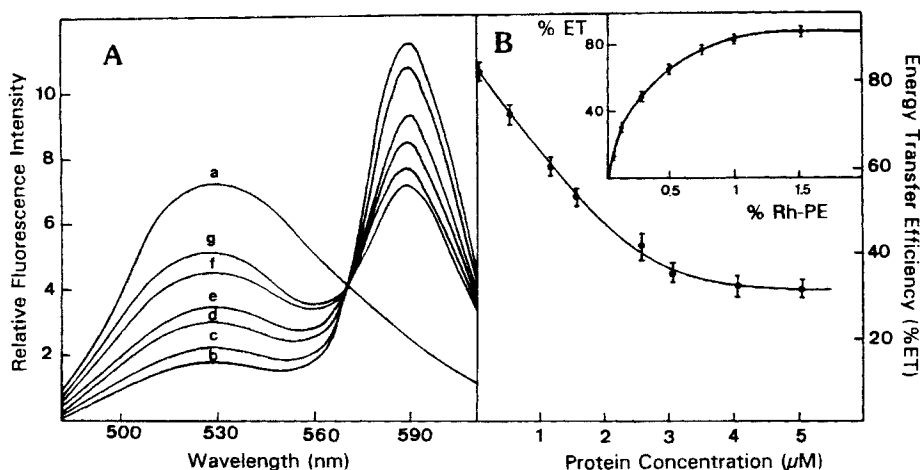


Fig. 4. Effect of *Sin a I* on the energy transfer between *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-Myr₂GroPEtn (donor) and *N*-(lissamine rhodamine B sulphonyl)-PtdEtn (acceptor) incorporated into Myr₂GroPGro vesicles. (A) Fluorescence intensity is expressed in arbitrary units. The phospholipid concentration is 150 μM in 50 mM Mops, pH 7.0, containing 0.1 M NaCl. Labelled vesicles were mixed with unlabelled vesicles (1:9 molar ratio). The spectrum (a) corresponds to labelled vesicles Myr₂GroPGro/*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-Myr₂GroPEtn (99:1 molar ratio) mixed with unlabelled vesicles (1:9). Spectrum (b) was obtained in the absence of protein [Myr₂GroPGro/*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-Myr₂GroPEtn /*N*-(lissamine rhodamine B sulphonyl)-PtdEtn 98:1:1 molar ratio]. Spectra (c–g) were the result of the incubation of Myr₂GroPGro (150 μM) with increasing amounts of *Sin a I* (0.5, 1.0, 1.5, 2.5, 4.0 μM, respectively). (B) Energy transfer efficiency (% ET) versus *Sin a I* concentration. Values are expressed as averages ± SD of three different experiments. The inset shows the efficiency of the donor-to-acceptor energy-transfer process versus the surface density of the acceptor (%Rh-PE), the amount of *N*-(lissamine rhodamine B sulphonyl)-PtdEtn (Rh-PE)/phospholipid molecule. Labelled Myr₂GroPGro vesicles and unlabelled vesicles were mixed at a 1:9 molar ratio.

recorded after 40 min of incubation. A plot of the energy transfer efficiency versus the *Sin a I* concentration is given in Fig. 4B. The energy transfer efficiency diminishes from approximately 80% to 30%, and a saturation plot is observed. This decrease is consistent with an approximately ninefold decrease of the acceptor surface density, based on the variation of the efficiency of the energy transfer process as a function of the number of energy acceptors/phospholipid molecule (Fig. 4).

The observed effect was not due to a potential instability of the vesicles. There were no changes in the energy transfer when labelled vesicles were incubated with *Sin a I* in the absence of unlabelled vesicles. Besides, no change in the efficiency of the energy transfer was observed when a mixture of fluorescence-labelled and unlabelled vesicles was incubated in the absence of the protein. The obtained results reveal a dilution of the fluorescence probes that indicates lipid mixing between labelled and unlabelled vesicles.

The experiments summarized in Fig. 4 have also been performed with Myr₂GroPCho vesicles. No change in the emission spectrum of a mixture of labelled and unlabelled vesicles was observed after addition of *Sin a I* to these zwitterionic vesicles (data not shown). This result agrees with results obtained from protein-binding and vesicle aggregation measurements when these vesicles were considered.

The lipid intermixing produced by *Sin a I* has also been observed by fluorescence depolarization measurements of 1,6-diphenyl-1,3,5-hexatriene-labelled vesicles. A mixture of Myr₂GroPGro and Pam₂GroPGro vesicles in a 1:1 molar proportion shows very different phase transition profiles in the presence and absence of the allergen (Fig. 5). In the absence of the protein, two different transition temperatures corresponding to each phospholipid, 23°C for Myr₂GroPGro and 36°C for Pam₂GroPGro, were observed. Addition of *Sin a I* to a mixture of independent Myr₂GroPGro and Pam₂GroPGro vesicles results in a lipid-protein complex which exhibits a single thermotropic transition (Fig. 5). In the pres-

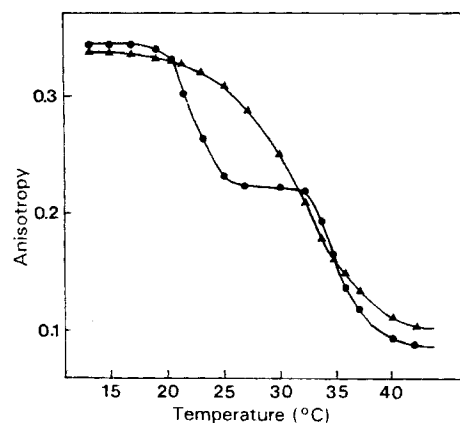


Fig. 5. Phase transition profiles of 1,6-diphenyl-1,3,5-hexatriene-labelled Myr₂GroPGro vesicles. Anisotropy values are the average of three experiments. Myr₂GroPGro vesicles plus Pam₂GroPGro vesicles in the absence (●) and in the presence (▲) of *Sin a I* at a 25:1 lipid/protein molar ratio. The total phospholipid concentration was 80 μg/ml in 50 mM Mops, pH 7.0, containing 0.1 M NaCl. Anisotropy was determined after equilibration of the samples for 10 min at each temperature.

ence of *Sin a I*, the temperature transition profile of the vesicle mixture loses its biphasic character as it corresponds to a single population of mixed Myr₂GroPGro/Pam₂GroPGro vesicles. This result also shows that the effect of the allergen on lipid mixing is not highly dependent on the acyl chain length of the phospholipid, in agreement with the aggregation data.

Release of vesicle aqueous contents

Sin a I promotes leakage of the vesicle aqueous contents, as determined from the observed dequenching of 8-aminonaphthalene-1,3,6-trisulfonic acid/*p*-xylylenebis(pyridinium)-

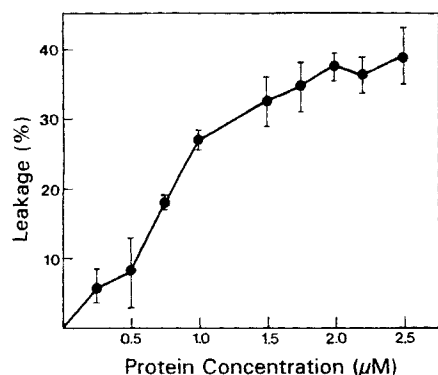


Fig. 6. Release of vesicle aqueous contents induced by *Sin a I*. Egg PtdGro vesicles (75 μ M), containing 8-aminonaphthalene-1,3,6-trisulfonic acid and *p*-xylylenebis(pyridinium)bromide encapsulated in the same vesicles, in 10 mM Tris, pH 7.4, containing 100 mM NaCl and 1 mM EDTA, were used. Results (averages $\pm$ SD of three different experiments) are expressed as the amount of leakage by considering the increase of the fluorescence emission of 8-aminonaphthalene-1,3,6-trisulfonic acid upon dequenching resulting from the vesicle leakage.

bromide encapsulated in the same vesicles upon addition of the allergen. The amount of leakage is calculated from the final fluorescence emission intensity of 8-aminonaphthalene-1,3,6-trisulfonic acid measured after approximately 20 min, the time required for equilibration of the process. A summary of the obtained results is given in Fig. 6. Egg PtdGro vesicles were used due to their higher encapsulation efficiency in comparison to that of synthetic phospholipid vesicles. The observed leakage of aqueous contents approaches saturation at about the same lipid/protein molar ratio observed in the lipid mixing process (Fig. 4B). Therefore, leaky structures are formed when *Sin a I* interacts with phospholipid vesicles.

DISCUSSION

The results demonstrate that *Sin a I*, the major allergen from table mustard, strongly interacts with phospholipid vesicles. The interaction would involve charge neutralization since acid phospholipids are required, which is in agreement with the cationic character of the protein. However, *Sin a I* perturbs the bilayers enough to produce lipid mixing between membranes and leakage of vesicle aqueous contents. Although acid bilayers appear to be rather uncommon under physiological circumstances, PtdGro is related to translocation of proteins across membranes [15–17]. In addition, the extracellular leaflet of the intestinal brush border membranes has larger amounts of acid phospholipids when compared to other plasma membranes [18]. Therefore, the interaction between *Sin a I* and phospholipid membranes described in this study may be underlying the allergenic character of this protein.

A food allergen must be an immunogenic protein capable of permeating the intestinal barrier [19]. Under conditions of increased permeability of the humoral barrier an enhanced allergic response has been observed [20]. The interaction of *Sin a I* with the epithelial gut membranes would allow an increased cellular capture of the protein and the subsequent access to the lymphoid tissues where the allergenic response is produced. Moreover, an interaction of *Sin a I* with membranes would minimize its elimination by secretory antibodies at the gut, since the time of exposure to their defensive

activity would be shortened. The interaction could also increase the immunogenicity of the protein at the local and systemic levels. It has been suggested [6] that the binding of a protein to lipid membranes would provide a multivalent antigenic structure to support the cross-linking of surface antibodies in the resting cells, a prerequisite in the T-cell-independent step of the B-cell activation and humoral immune response [21]. The interaction of *Sin a I* with membranes may also increase its survival in the blood stream as a protective mechanism against proteolytic digestion of the allergen and clearance [6]. In this respect, melittin, a major allergen (*Api m I*) from bee venom, also interacts with lipid membranes [22, 23], and a correlation between the biophysical basis of its IgG and IgE immunogenicity and its membrane binding has been proposed [6].

Based on the obtained results, we can speculate that a food allergen is a protein resistant to the proteolysis involved in food digestion (*Sin a I* is highly resistant to 24-h extensive treatments with trypsin, chymotrypsin or pepsin; data not shown), with an ability to interact with membranes. This could result in (a) increased cellular uptake, (b) reduced neutralization by secretory antibodies, (c) increased permanence in the blood stream, and (d) enhanced reaction with IgE through the epitopes present in the polypeptide molecule.

Finally, antifungal and antimicrobial activities have been found to be associated with 2S albumins from radish and rapeseed [24, 25]. Similar activities might be attributed to *Sin a I* since radish, rapeseed and mustard belong to the same Brassicaceae family and their 2S albumins have a very high degree of sequence similarity (more than 90%) [4, 26]. The molecular basis of these antifungal and antibacterial activities are unknown but they may be related to the ability of these proteins to interact with membranes as demonstrated in this study.

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