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Dual specificity of sterol-mediated glycoalkaloid induced membrane disruption

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In this study the effects of the glycoalkaloids α-solanine, α-chaconine, α-tomatine and the aglycone solanidine on model membranes composed of PC in the absence and presence of sterols have been analysed via permeability measurements and different biophysical methods. The main result is that glycoalkaloids are able to interact strongly with sterol containing membranes thereby causing membrane disruption in a way which is specific for the type of glycoalkaloid and sterol. For this dual specificity both the sugar moiety of the glycoalkaloid and the side-chain of the sterol on position 24 turned out to be of major importance for the membrane disrupting activity. The order of potency of the glycoalkaloids was α-tomatine > α-chaconine > α-solanine. The plant sterols β-sitosterol and fucosterol showed higher affinity for glycoalkaloids as compared to cholesterol and ergosterol. The mode of action of the glycoalkaloids is proposed to consist of three main steps: (1) Insertion of the aglycone part in the bilayer. (2) Complex formation of the glycoalkaloid with the sterols present. (3) Rearrangement of the membrane caused by the formation of a network of sterol-glycoalkaloid complexes resulting in a transient disruption of the bilayer during which leakage occurs.

Introduction

Glycoalkaloids, or steroidal glycoalkaloids, are natural toxins occurring in solanaceous species like potato and tomato which are thought to be partly responsible for the natural defense of these crops against diseases and predation. Toxicity of these compounds to a wide range of organisms including humans has been observed [1,2].

In the cultivated potato (*Solanum tuberosum* L.) mainly two glycoalkaloids, α-solanine and α-chaconine, are found. They consist of the same aglycone, solanidine, but differ in their sugar moiety (Fig. 1). From tomato (*Lycopersicon esculentum*) one glycoalkaloid, α-tomatine, has been isolated. It consists of the aglycone tomatidine and a tetrasaccharide (Fig. 1).

The mechanism whereby these glycoalkaloids cause the toxic effects is still unknown. The glycoside is generally considered to be the active form but this has been questioned by Segal and co-workers [3–5], who concluded that the aglycone, liberated by surface glycosidases, is the active moiety. However, Roddick and co-workers [6,7] showed evidence in support of the glycoside as the active form, the membrane being the target. The presence of sterols in the membrane turned out to be a prerequisite for these glycoalkaloids to exert an effect in vitro. Using peroxidase loaded lipid vesicles membrane disruption was observed by measuring peroxidase leakage from those vesicles [6,7]. Membrane disrupting effects were found using fungal protoplasts [8], rabbit erythrocytes [8], red beet cells [8] and mouse cell lines [9]. Comparable effects have also been observed for the saponin digitonin, a compound with a structure closely related to glycoalkaloids [10].

The purpose of this study was to investigate the interaction of these glycoalkaloids with membranes in more detail using lipid vesicles as model systems. Evidence is provided that glycoalkaloids irreversibly bind to membrane sterols causing a rapid loss of barrier function. This membrane disruptive effect turned out to be dependent on: (1) the composition of the sugar moiety of the glycoalkaloid and (2) the type and con-

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Abbreviations: PC, egg yolk phosphatidylcholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; CF, 6-carboxyfluorescein; DMF, dimethylformamide; TLC, thin-layer chromatography; DSC, differential scanning calorimetry; MLV, multilamellar vesicles.

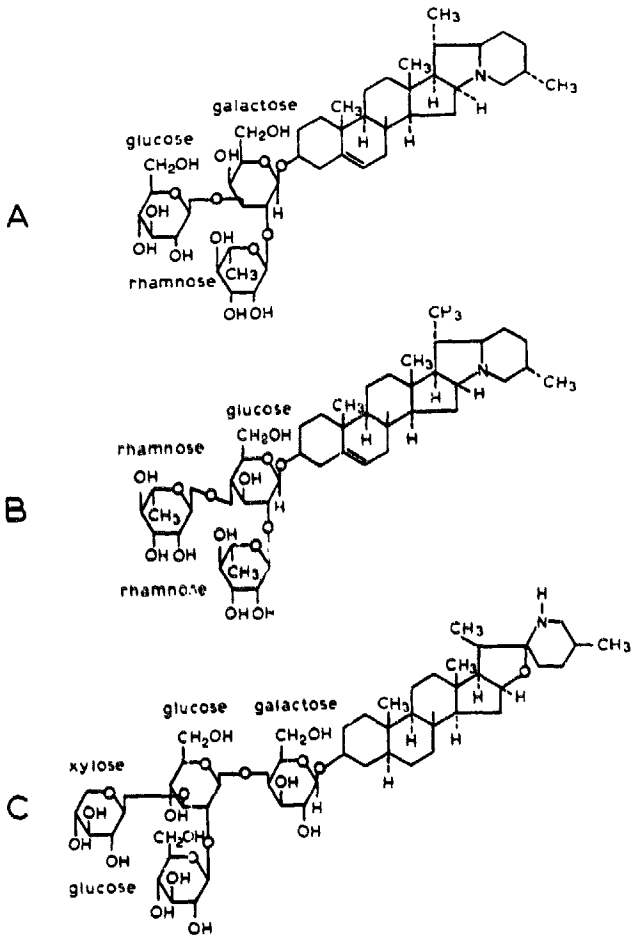

Fig. 1. The structures of the glycoalkaloids from potato (α-solanine (A) and α-chaconine (B)) and tomato (α-tomatine (C)).

tent of sterol present in the membrane. A model for the mechanism of glycoalkaloid-induced membrane disruption is proposed.

Materials and Methods

Materials

α-Chaconine, α-solanine, α-tomatine, solanidine, fucosterol and β-sitosterol were obtained from Sigma (USA). Cholesterol and ergosterol were obtained from Merck (Germany) and ICN (USA), respectively. Egg-yolk phosphatidylcholine (PC) was isolated and purified as described by Van Duijn et al. [11], whereas 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was synthesized according to the procedure of Van Deenen and De Haas [12]. 6-Carboxyfluorescein (Eastman Kodak, USA) was purified by active carbon treatment, recrystallisation from water/ethanol (2:1, v/v) and Sephadex LH20 (Pharmacia) column chromatography [13]. The glycoalkaloids were dissolved in dimethylformamide (DMF) up to 20 mM, whereas solanidine was dissolved in methanol up to 10 mM. The dissolved glycoalkaloids were regularly tested for possible hydrolysis or breakdown by TLC using methanol/chloroform/1% ammonium chloride (in H_2O) (50:50:1, v/v) as the solvent. All reagents were analytically pure and solvents used were of analytical grade.

Vesicle preparation

Large unilamellar vesicles were prepared from egg-yolk PC and sterols (total lipid 10 μmol) in 1 ml buffer using the extrusion technique (0.4 μm polycarbonate filters, Nuclepore) described by Mayer et al. [14]. Phospholipids were quantified after perchloric acid destruction, by the method of Fiske and SubbaRow [15]. All vesicle experiments were carried out at room temperature, unless otherwise stated.

CF leakage assay

Leakage of 6-carboxyfluorescein (CF) from loaded vesicles was used to detect membrane disruptive effects of glycoalkaloids. CF was loaded by preparing vesicles in 1 ml of 10 mM Hepes buffer (pH 7.4) containing 40 mM CF, 85 mM NaCl and 1 mM EDTA. Free CF was removed by gel-filtration over a Sephadex G-75 column (1 × 30 cm; Pharmacia), eluted with a 10 mM Hepes buffer (pH 7.4) containing 140 mM NaCl and 1 mM EDTA. The additional NaCl was used to compensate for the osmotic pressure of the CF inside the vesicles. 20 μl vesicle suspension (approx. 150 nmol lipid) were thoroughly mixed with 1.96 ml elution buffer in a 3 ml fluorescence cuvet. After 1 min, 20 μl of a glycoalkaloid solution was added. Fluorescence was measured on a SLM/Aminco SPF-500C spectrofluorometer at an excitation of 430 nm monitoring CF release as an increase in emission intensity at 513 nm, due to dequenching of CF fluorescence [16]. Total amount of CF loaded was determined by lysing the vesicles with 20 μl 10% (v/v) Triton X-100. The release was expressed as:

$$\% \text{ leakage} = ((F_t - F_0)/(F_T - F_0)) \times 100$$

F_0 is the initial fluorescence, F_t is the fluorescence after an incubation period of t minutes and F_T the fluorescence after addition of Triton X-100. CF leakage was monitored for 5 min, after which leakage had almost completely ceased.

Binding experiments

For binding experiments PC vesicles containing 0 or 50 (mol)% cholesterol were prepared in a 10 mM Hepes buffer (pH 7.4) containing 140 mM NaCl and 1 mM EDTA. 30 μl vesicle suspension (approx. 225 nmol lipid) was mixed with 2.94 ml of the same buffer. Subsequently, 30 μl of a glycoalkaloid solution was added, and after an incubation of 10 min these samples were centrifuged (4°C, 45 min at 541 000 × *g*) in a Beckman optima TL table centrifuge (rotor TLA 100.3).

Non-bound glycoalkaloids in the supernatant were determined using the CF leakage assay. To 1.98 ml supernatant 20 μl of a CF-containing vesicle suspension was added and the fluorescence monitored. The CF-loaded vesicles consisted of PC and either 50% cholesterol or 50% β-sitosterol for α-chaconine and α-solanine quantification, respectively. When this procedure was applied to vesicle free samples it could be demonstrated that the supernatant had a similar CF releasing capacity as the non-centrifuged samples demonstrating that the free glycoalkaloids did not pellet during centrifugation.

Differential scanning calorimetry (DSC)

The effect of the glycoalkaloids on the gel → liquid-crystalline phase transition of aqueous DPPC dispersions was studied by DSC (Perkin-Elmer, DSC-4). Samples were prepared in two different ways. Firstly, multilamellar vesicles (MLV; 10 μmol of lipid) were prepared by mechanically dispersing a dry film of DPPC or DPPC/cholesterol (3:1, molar ratio), dried from chloroform, in 2 ml elution buffer whereafter 125 μl 20 mM (2.5 μmol) glycoalkaloid solution was added. Secondly, MLV were prepared similarly but now from mixed films of DPPC, cholesterol and glycoalkaloid in the same ratio. As controls PC or PC/cholesterol MLV were used to which 125 μl DMF was added. After 10 min, the lipids were pelleted by centrifugation (4°C, 15 min a 40000 × g) and part of the pellets were transferred into a 17 μl volatile aluminium sample pan. Thermograms were obtained using a scan rate of 5 C°/min. All samples were scanned at least two times, yielding identical thermograms. After measurement the pans were opened and the phospholipid content determined [15]. Enthalpies were calculated by integrating peak areas and relating them to the total amount of phospholipid in the pans. The calorimeter was calibrated using the enthalpy of the gel to liquid-crystalline phase transition of DPPC (8.0 kcal/mol [17]) as a reference.

Monolayer studies

Monolayer experiments were carried out at room temperature using a round teflon trough (3.0 × 0.5 cm). Surface pressure was measured with an electrochemically roughened platinum (Wilhelmy) plate (2 cm wide) and an electrobalance (Beckman LM 500). The subphase consisted of 5 ml 10 mM Tris-HCl (pH 7.4) and lipids were spread from chloroform until a surface pressure of approximatly 20 mN/m was reached. Then 25 μl of a glycoalkaloid solution were added to the aqueous sub-phase and the increase of surface pressure was monitored during the first 5 min after addition.

Freeze-fracture electron microscopy

For freeze-fracturing PC/cholesterol (1 : 1, mol/mol) vesicles were prepared in 1.5 ml 10 mM Hepes buffer (pH 7.4) containing 140 mM NaCl and 1 mM EDTA. Next, 0.5 ml vesicle suspension (3.5 μmol lipid) was added to 2.5 ml buffer and mixed. After 1 min 87.5 μl of a 20 mM glycoalkaloid solution was added. To the control vesicles 87.5 μl DMF was added. After 10 min incubation time the samples were cen-

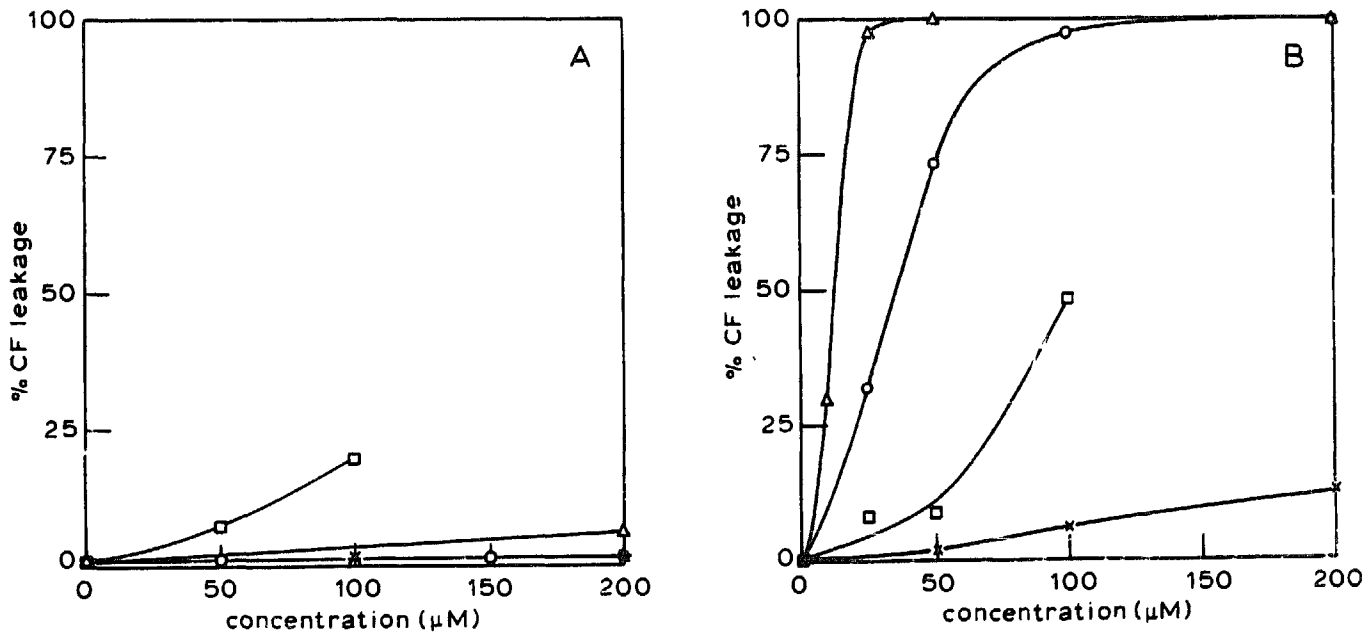

Fig. 2. Glycoalkaloid induced CF leakage from (A) PC and (B) PC/cholesterol (1:1, molar ratio) vesicles. CF leakage was measured 5 min after addition of different amounts of α-solanine (×), α-chaconine (○), α-tomatine (△) or solanidine (□) to the vesicles. Each point represents the average of six replicates with a standard deviation of less than 2%.

trifuged (4°C, 45 min at 541000 × g), and the pellets were supplemented by 30% (v/v) glycerol as cryoprotectant and quenched in solid-liquid N_2. The samples were subsequently fractured in a Balzers freeze-etch machine according to standard procedures. Replicas were examined in a Philips CM-10 electron microscope.

Results

Leakage experiments

To investigate the membrane disruptive effect of the glycoalkaloids, these compounds were tested in the carboxyfluorescein (CF) leakage assay. In the absence of sterols the tested glycoalkaloids caused no (α-solanine and α-chaconine) or a very minor (α-tomatine) CF leakage up to a concentration of 200 μM (Fig. 2A). However, the aglycon solanidine caused up to 20% CF leakage at a concentration of 100 μM. Higher concentrations of solanidine could not be tested because of solubility problems. On the contrary, using PC vesicles containing 50 mol% cholesterol, considerable leakage was observed (Fig. 2B). α-Tomatine and α-chaconine caused complete CF leakage at concentrations of 50 and 100 μM, respectively. α-Solanine only caused minor leakage compared to α-chaconine, pointing out an important role for the sugar moiety in glycoalkaloid induced membrane leakage. The aglycone solanidine was somewhat more effective compared to the effect on pure PC vesicles but considerably less compared to the effects of α-chaconine and α-tomatine. These observations and the high solanidine induced leakage from pure PC vesicles indicated a different type of interaction of this compound with membranes as compared to glycoalkaloids. The order of potency of inducing CF leakage from PC/cholesterol vesicles at equimolar concentrations was α-tomatine > α-chaconine > solanidine > α-solanine.

The leakage process turned out to be very fast as illustrated for α-chaconine in Fig. 3; most of the CF was released from the vesicles during the first minute after addition and reached steady state levels after 5 min. These levels were proportional to the amount of α-chaconine added. Such behaviour can in principle arise from two different situations, either from complete release of CF from part of the vesicles or partial CF release from all vesicles. The former case could be caused by local high concentrations of the glycoalkaloid directly after addition. To get insight into this possibility the order of addition was changed. When 20 μl glycoalkaloid solution was added to the buffer and mixed for 1 minute before subsequent addition of the vesicles this resulted in similar CF leakage kinetics (data not shown). This indicated that the observed curve was not due to mixing problems and that it is likely that all vesicles partly release CF. Although we favour the latter explanation, it cannot be excluded that due to inhomogeneity of the vesicle suspension a difference in susceptibility exists.

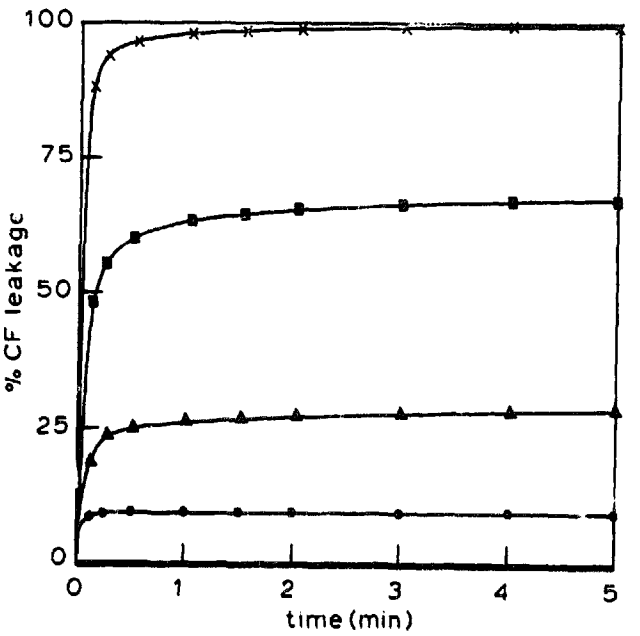

Fig. 3. Leakage of CF from PC/cholesterol (1:1) vesicles with time induced by 100 μM (×), 50 μM (■), 25 μM (▲) or 10 μM (●) α-chaconine. Each point represents the average of six replicates with a standard deviation of less than 2%.

The presence of cholesterol appeared to be a prerequisite for the glycoalkaloid induced membrane leakage. In order to study the cholesterol dependency of the leakage process the cholesterol content of the vesicles was varied. Both α-chaconine (Fig. 4A) and α-tomatine (Fig. 4B) caused increased leakage with increasing cholesterol content. As could be expected, α-solanine only caused minor leakage at all cholesterol contents (data not shown). For α-chaconine a minimum cholesterol content of approx. 10% appeared to be neccesary to cause significant CF leakage. For α-tomatine, the minimum cholesterol content causing CF leakage could not be detected because some minor leakage was already observed without cholesterol (Fig. 4A).

Since glycoalkaloids have been reported to affect various biological membranes [8] and different sterols can be found in different membranes, the sterol specificity of the membrane disrupting effect was studied. Four different sterols were used (Fig. 5); cholesterol, the fungal ergosterol and the plant sterols β-sitosterol and fucosterol. Interestingly, large differences in CF leakage from vesicles containing different sterols occurred. For α-solanine, the presence of the ergosterol in the membrane resulted in similar low leakage compared to cholesterol, whereas leakage from vesicles containing β-sitosterol or fucosterol was very high (Fig. 6A). α-Chaconine induced leakage was high for all

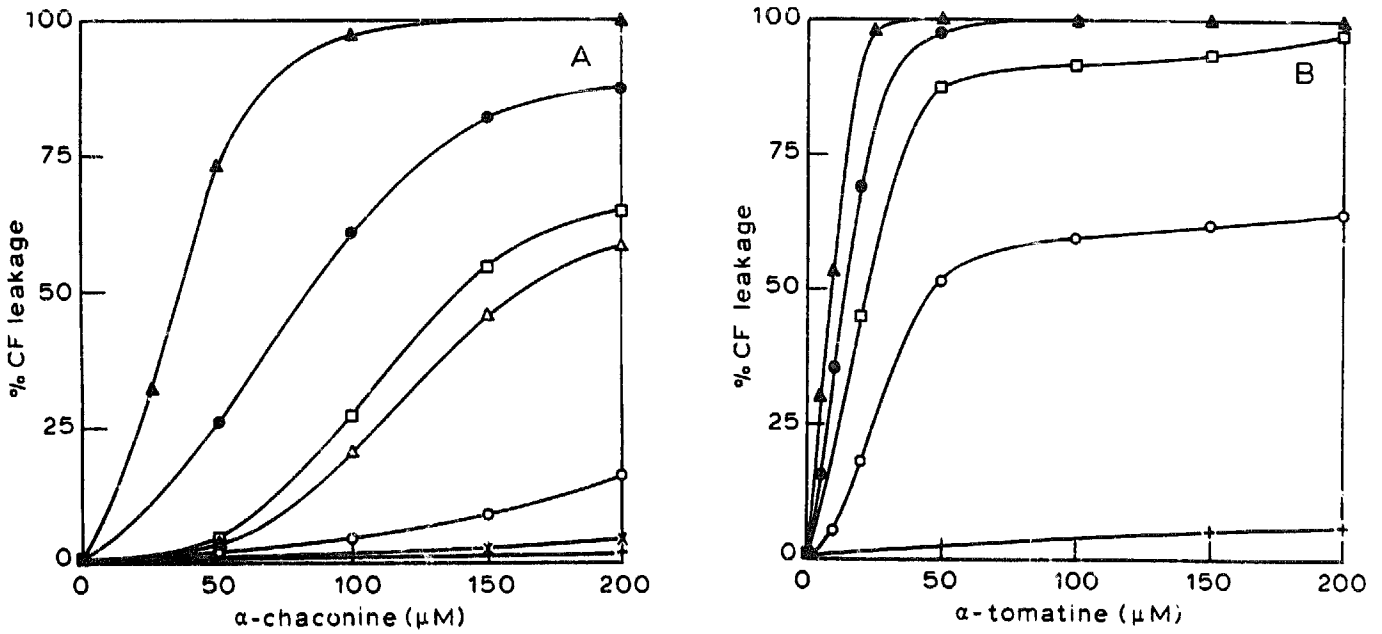

Fig. 4. Sterol content dependent CF leakage from PC vesicles induced by α-chaconine (A) or α-tomatine (B) measured 5 min after the addition of the glycoalkaloid. Vesicles contained 0% (+), 9% (×), 13% (○), 17% (△), 23% (□), 33% (●) or 50% (▲) cholesterol. Each point represents the average of six replicates with a standard deviation of less than 2%.

tested sterols (Fig. 6B) and in all cases higher than α-solanine induced leakage. Nevertheless the order of efficiency of the different sterols to mediate CF leakage was similar for both compounds. These data demonstrated that glycoalkaloid induced CF leakage is strongly dependent on the type of sterol present in the membrane.

Binding experiments

To analyse whether the differences between α-chaconine and α-solanine in efficiency to cause CF release from cholesterol containing vesicles are due to differences in membrane affinity, binding experiments were performed (Table I). α-Chaconine did bind efficiently to the PC/cholesterol (1 : 1) vesicles which was largly due to the presence of sterol as much less binding was observed for vesicles devoid of sterol. In contrast, α-solanine showed much less efficient binding to the cholesterol containing vesicles than α-chaconine suggesting that the differences in ability to cause CF release are due to differences in efficiency of binding of the two glycoalkaloids to the vesicles.

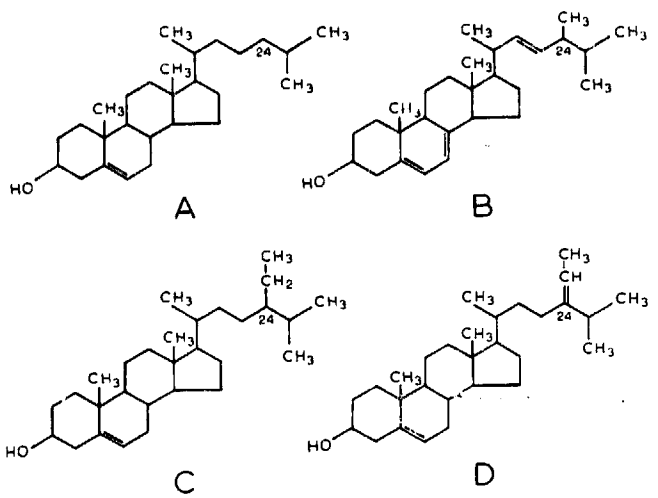

Fig. 5. The structures of cholesterol (A), ergosterol (B), β-sitosterol (C) and fucosterol (D).

To examine whether the observed α-chaconine binding to lipid vesicles was reversible, additional CF leakage experiments were carried out using both CF containing and empty vesicles. Fig. 7A shows that subsequential additions of CF containing PC/cholesterol (1 : 1, molar ratio) vesicles (50 nmol lipid) to a 50 μM α-chaconine solution results in stepwise CF release from the vesicles such that finally a CF leakage was observed similar to that observed when all vesicles were added at $t = 0$. The amount of CF leakage was decreasing with each vesicle addition. If, however, one (Fig. 7B) or two (Fig. 7C) batches of 50 nmol empty PC/cholesterol vesicles were added before the addition of CF-loaded PC/cholesterol vesicles (Figs. 7B and C), the subsequent CF leakage did not reach the level of the first addition in experiment 7A but reached instead that of the second or third addition, respectively. Also, the total amount of leakage was not identical to the amount of leakage obtained when all CF-loaded vesicles were added at $t = 0$. This strongly indicated that the α-chaconine once bound to the empty cholesterol containing vesicles is no longer available to cause leakage from newly added vesicles. Adding empty PC/cholesterol vesicles after CF-loaded PC/choles-

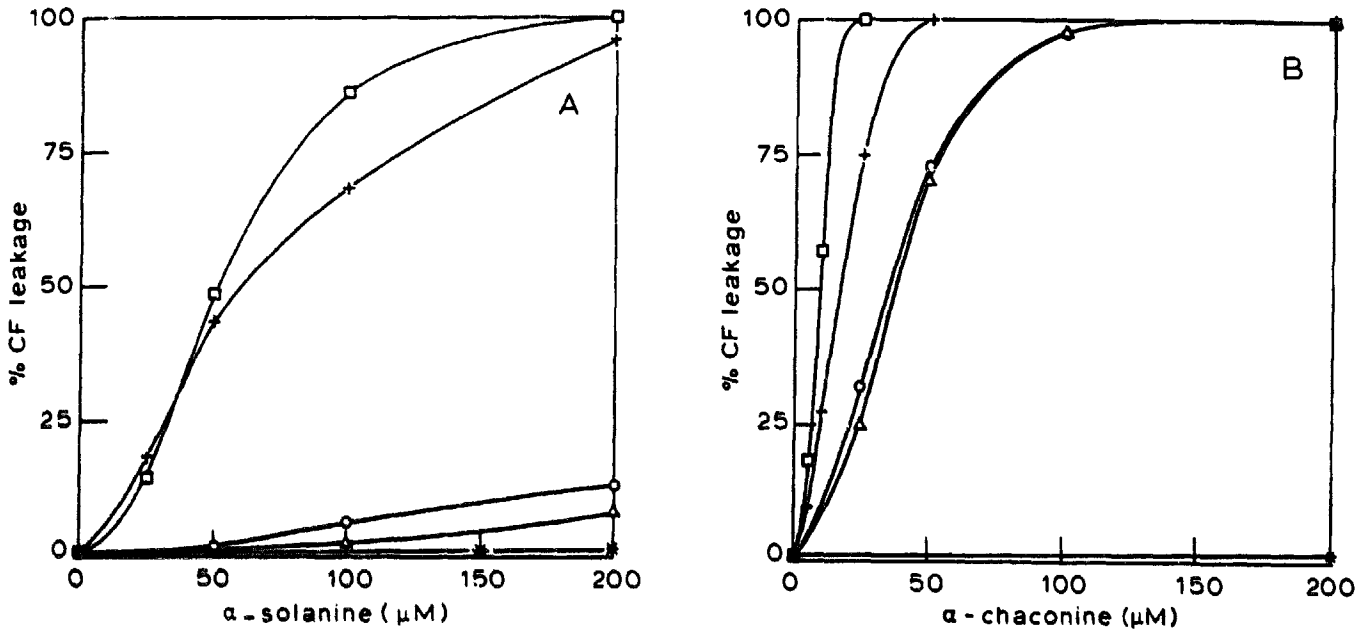

Fig. 6. Sterol specificity of α-solanine (A) and α-chaconine (B) induced CF leakage. Leakage from PC vesicles containing no sterol (*), 50% ergosterol (△), 50% cholesterol (○), 50% β-sitosterol (+) or 50% fucosterol (□) was measured 5 min after the addition of the glycoalkaloid. Each point represents the average of six replicates with a standard deviation of less than 2%.

terol vesicles did not result in increased leakage (data not shown), meaning that all additional leakage observed after each addition originates from the newly added vesicles.

Monolayer studies

To check whether α-solanine, α-chaconine and their aglycone solanidine insert in between the lipid molecules, monolayer experiments were carried out. The collapse pressure of α-solanine, α-chaconine and solanidine was found to be less than 2 mN/m, indicating that these compounds are not by themselves interface seeking at the starting pressure of 20 mN/m used in the experiments with a lipid monolayer. Table II shows that all compounds caused a pressure increase which was larger than that observed when only the solvent (DMF) used to dilute the compounds was added. The highest increase for all compounds was observed with the PC/cholesterol monolayer, nevertheless they also caused a considerable increase in pressure for the PC monolayer in particular in the case of solanidine. Interestingly, the order of potency is similar to the order obtained in the CF leakage assay. The pressure increases imply that the glycoalkaloids insert in between the acyl chains of the lipids which process is facilitated by cholesterol.

Differential scanning calorimetry

To analyse whether α-solanine and α-chaconine interact with membrane cholesterol in such a way that

TABLE I

Binding of α-solanine and α-chaconine to vesicles consisting of PC or PC/cholesterol

PC or PC/cholesterol vesicles (225 nmol lipid) were incubated with various concentrations of glycoalkaloids, whereafter they were centrifuged (4°C, 45 min at 541000 × g) and non-bound glycoalkaloid was determined using the CF leakage assay. Data represent means ± S.D. for three determinations.

Glycoalkaloid (μM)		% bound to vesicles	
		PC	PC/chol 1:1
α-Solanine	25	0±1	2±1
	50	2±1	12±2
	100	3±3	14±2
α-Chaconine	25	8±2	72±2
	50	4±4	68±4
	100	15±3	75±2

TABLE II

Effect of α-solanine, α-chaconine and solanidine on monolayer surface pressure

Monolayers of lipids were spread on an aqueous phase up to a surface pressure of 20 mN/m. Subsequently, 25 μl of a glycoalkaloid solution were added to the aqueous sub-phase and the increase of surface pressure determined after 5 min. Data represent means ± S.D. for three determinations.

	Δ surface pressure π (mN/m)	
	PC	PC/chol 1:1
DMF (control)	2.8±0.0	2.5±0.3
α-Solanine	5.8±0.2	10.5±0.1
α-Chaconine	8.2±0.2	18.7±0.1
Solanidine	11.5±0.1	17.2±0.2

the sterol is removed from its interaction with the fatty acyl chains, we performed (DSC) experiments on DPPC membranes. Samples were prepared in two different ways. Either, α-solanine and α-chaconine were added to preformed multilamellar vesicles (MLV) similar to the procedure used in the leakage assay or MLV were prepared from mixed films of lipids and glycoalkaloids allowing maximal interaction between the molecules.

The presence of α-solanine hardly influenced the pre and main phase transition of DPPC in both protocols (Fig. 8; curves 2 and 3). Only a slight decrease in main transition temperature was observed, whereas the energy content of the phase transition did not change significantly (Table III). The thermotropic properties of the control DPPC samples were in good agreement with the literature. In DPPC vesicles containing 25% cholesterol the phase transition was virtually absent due to the liquidifying effect of cholesterol (Fig. 8; curve 4). Addition of α-solanine to the cholesterol containing sample only had a minor effect on the thermogram (Fig. 8; curves 5 and 6) resulting in only a slight increase in energy content for the second preparation (Table III) in agreement with the lower efficiency of interaction of this glycoalkaloid with cholesterol containing model membranes. However, α-chaconine caused considerable effects on the main transition both in pure DPPC vesicles as well as in the cholesterol containing samples. The presence of the glycoalkaloids shifted the main transition to an onset temperature of 37°C which effect was complete for the sample prepared out of mixed films (Fig. 8; curves 2 and 3). α-Chaconine had only a minor effect on the energy content of the phase transition (Table III). More interestingly, α-chaconine partially restored the

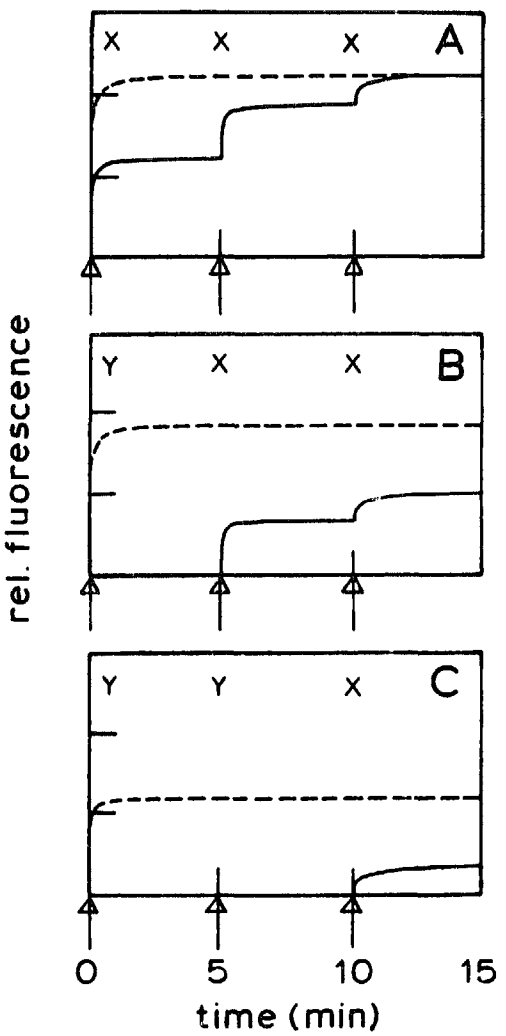

Fig. 7. Analysis of the reversibility of α-chaconine binding to vesicles. At 0, 5 and 10 min vesicles (50 nmol total lipid) were added (arrows) to a 50 μM α-chaconine solution in buffer. Vesicle suspensions were: X, PC/cholesterol (1:1) CF-loaded. Y, PC unloaded. Dotted lines indicate the leakage curves obtained when the total amount of CF-loaded vesicles was added at $t = 0$. Experiments were carried out in triplicate.

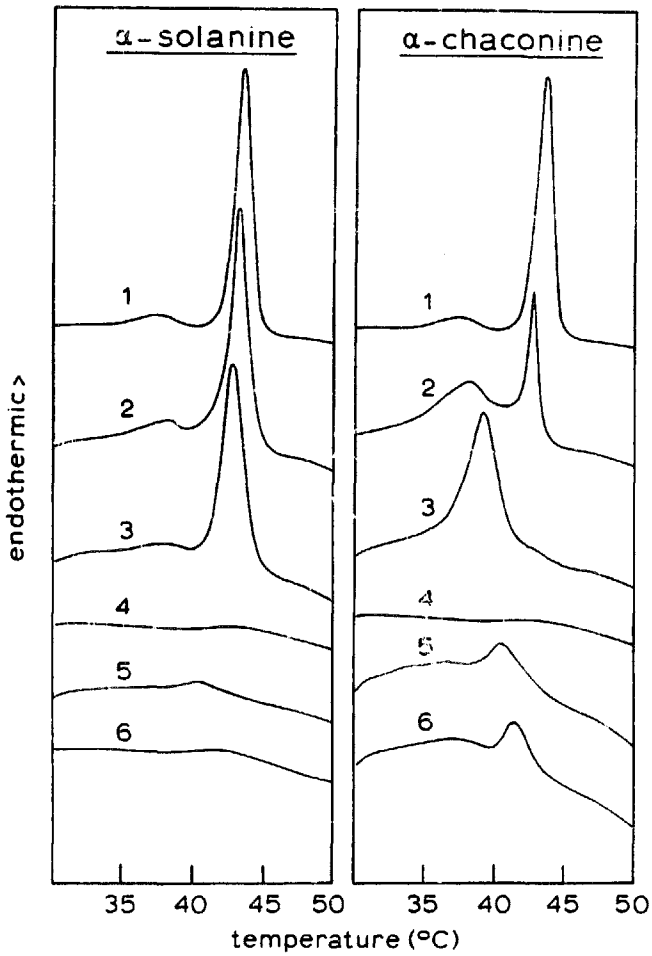

Fig. 8. The effect of α-solanine and α-chaconine on DSC thermograms (heating curves) of model membranes consisting of DPPC (thermogram 1–3) and DPPC/cholesterol (3:1, molar ratio) (thermogram 4–6). 1 and 4 are controls. In 2 and 5 the glycoalkaloids were added to preformed MLV, in 3 and 6 they were pre-incorporated into MLV. For details see the experimental section.

TABLE III

The effect of α-solanine and α-chaconine on the energy of the phase transition of DPPC and DPPC/cholesterol vesicles

The controls were vesicles incubated with DMF only. The glycoalkaloids were added to DPPC and DPPC/cholesterol after MLV preparation or included in MLV preparation. After 10 min the lipids were centrifuged (4°C, 15 min at 40000× *g*) and the pellets were used for a DSC scan (5 C°/min). Enthalpies were calculated from the thermograms (see Fig. 8) by integrating peak areas and relating them to the total amount of phospholipids used. Data represent means ± S.D. for three determinations.

Sample	Enthalpy of phase transition (kcal/mol)	
	DPPC	DPPC/chol. 3:1
Added to MLV:		
Control	8.0 ± 0.02	0.4 ± 0.04
α-Solanine	7.8 ± 0.09	0.4 ± 0.04
α-Chaconine	8.1 ± 0.13 [a]	1.5 ± 0.02
Prepared in MLV:		
Control	8.0 ± 0.02	0.4 ± 0.04
α-Solanine	7.9 ± 0.02	0.7 ± 0.01
α-Chaconine	7.3 ± 0.12	2.3 ± 0.03

[a] Sum of both peaks.

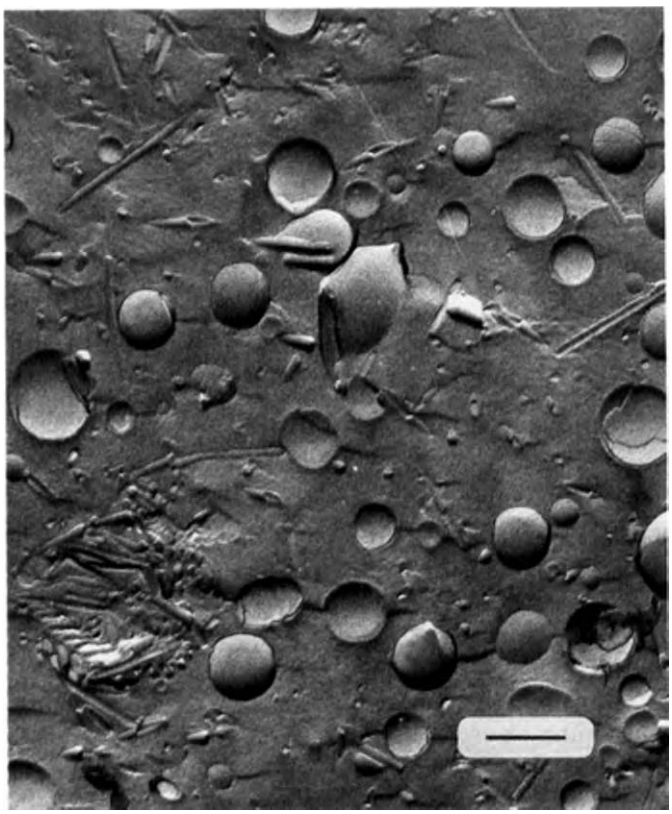

Fig. 9. Freeze-fracture electron micrograph (50000×) of a sample of unilamellar PC/cholesterol (1:1, molar ratio) vesicles after the addition of α-chaconine in a cholesterol/glycoalkaloid ratio of 1. 10 min after the addition of α-chaconine the sample was centrifuged (4°C, 541000× *g* for 45 min) and the pellet was supplemented by 30% (v/v) glycerol as cryoprotectant and quenched in solid-liquid N_2. The bar corresponds to 0.20 μm.

phase transition in DPPC/cholesterol membranes (Fig. 8; curves 5 and 6, Table III), with the maximum effect for the mixed film sample. These results indicate formation of an α-chaconine-cholesterol complex in which the cholesterol molecules are shielded from interaction with the acyl chains of the DPPC.

Freeze-fracture electron microscopy

For freeze-fracture electron microscopy α-solanine or α-chaconine were added to PC/cholesterol vesicles (1:1). The electronmicrographs showed no effect of α-solanine on vesicle stucture (in a 1:1 molar ratio to cholesterol) compared to control vesicles. In both cases smooth fracture faces of mainly unilamellar vesicles were observed (data not shown). However, α-chaconine addition in the same ratio resulted in major effects (Fig. 9). There were hemitubular structures present in the sample and in some cases such structures could be seen budding from the vesicles. Comparing these results with the experiments carried out with digitonin [18], a saponin with a similar molecular structure to the glycoalkaloids, it seems likely that these tubular structures are cholesterol-α-chaconine complexes, which are formed through phase-separation of these components in the membrane.

Discussion

In this study we analysed with different techniques the effects of the glycoalkaloids α-solanine, α-chaconine, α-tomatine and the aglycone solanidine on model membranes composed of PC in the absence and presence of different sterols. The main result is that these compounds are able to strongly interact with sterol containing membranes in a way which is specific for the type of glycoalkaloid and sterol. Before addressing this dual specifity we would like to propose and discuss a model of the mode of membrane interaction of α-chaconine. The model describes a mechanism consisting of several successive steps, the first one being association of the molecule with the bilayer. Monolayer, DSC and binding experiments show that α-chaconine has a weak but significant affinity for pure PC bilayers which can result in membrane penetration, most likely of the aglycone part of the molecule. However, this does not result in loss of barrier function. The presence of cholesterol greatly increases the affinity of α-chaconine for the bilayer and results in increased insertion of the aglycone moiety in between the lipid molecules. Above a threshold concentration of sterols in the membrane (± 10 mol%) a stable α-chaconine-cholesterol complex is formed in which the cholesterol molecule is shielded from its interaction with the acyl chains of PC. This complex possibly corresponds to the cylindrical structures observed by EM in the vesicle preparation. The formation of the

chaconine-cholesterol complex is responsible for a loss of bilayer integrity which could arise from different mechanisms. The present data favour a transient leakage possibly associated with budding of these tubular structures.

The presence of sterols in the membrane appears to be an absolute requirement for the membrane disruptive action of the glycoalkaloids. Although glycoalkaloids bind to vesicles and insert into monolayers composed of pure phosphatidylcholine, the presence of cholesterol in the membrane greatly facilitates both types of interaction. The increased affinity of glycoalkaloids for cholesterol containing membranes is partly due to the carbohydrate moiety of the glycoalkaloids as the aglycone solanidine inserted less efficiently in such monolayers than α-chaconine. Glycoalkaloid binding to PC/cholesterol vesicles appeared to be irreversible, while binding to cholesterol free membranes may be a more dynamic equilibrium between monomeric glycoalkaloids in the aqueous and lipid phase.

Membrane vesicles containing less than approximately 10 mol% cholesterol were completely insensitive to α-chaconine even at high concentrations of the glycoalkaloid, indicating that a certain density of the glycoalkaloid-cholesterol complex is required to cause membrane disruption. Above this sterol threshold concentration vesicles become highly sensitive to glycoalkaloids. Then stable glycoalkaloid-cholesterol complexes are formed, which may cause lateral phase separation into domains rich in glycoalkaloid and cholesterol and cholesterol-free domains. The initial glycoalkaloid-sterol interaction is probably via the aglycone part of the glycoalkaloid and the sterol ring system. However, the fact that the aglycone solanidine does not cause as much leakage as α-chaconine could be explained by the lack of intermolecular sugar–sugar interactions in that case.

A high specificity in membrane disruptive activity of the three glycoalkaloids tested exists. The higher potency of α-tomatine could be caused by its different steroid structure compared to α-chaconine and α-solanine which share the same aglycone. However, the additional monosaccharide in its carbohydrate moiety seems to be a more likely explanation as Nishikawa et al. [10] demonstrated for the structurally related saponin digitonin and digitonin analogs. With these compounds membrane disruption increased with increasing size of the sugar moiety. In addition, we showed in this study that even smaller differences than the carbohydrate moiety size of glycoalkaloids can have dramatic effects. In view of the fact that α-chaconine and α-solanine contain both a trisaccharide group, but with different composition, it is even more remarkable that large differences are observed for both compounds in membrane binding, insertion and disruption. These differences could either be explained by subtle changes in the sugar moiety volume or by the different side chains on the sugar ring structures which influence the sugar–sugar intermolecular interactions and thereby the formation of stable glycoalkaloid-sterol complexes.

Interestingly, the membrane disruptive activity of glycoalkaloids is also highly specific for the type of sterol present in the membrane. α-Solanine induced leakage of ergosterol containing membranes is at the same low level as for cholesterol containing membranes, but in the presence of the plant sterols β-sitosterol or fucosterol high leakage occurred. A similar tendency was observed for α-chaconine which is also more active with plant sterol containing membranes than with membranes containing cholesterol or ergosterol although the differences are less pronounced compared to α-solanine. The major structural difference between the two groups of sterols is the size of the moiety at C_{24} in the sterol side chain which consists of two C-atoms in the case of the plant sterols. Therefore, the observed effects with different sterols can be interpreted on the basis of steric factors that alter the interaction between the membrane embedded sterol part and the aglycone part of the glycoalkaloids.

Cholesterol appears to be the target for many other toxins [19] other than glycoalkaloids and saponins. In addition, polyene antibiotics [20] and bacterial toxins [21,22] induce membrane leakage by complex formation with sterols whereby either pores are formed or membrane disruption occurs. In the case of glycoalkaloid-induced membrane leakage, (transient) lysis is involved since also larger molecules than carboxyfluorescein, the tested compound in this study, leak through membranes [6,23].

Comparing the glycoalkaloid action on model membranes described in this study with glycoalkaloid effects on biological membranes [8,9,24] it seems likely that the observed effects on biological membranes are the consequences of events described in the presented model. Firstly, in all studies α-chaconine turned out to be more potent than α-solanine and secondly, glycoalkaloid induced leakage only occurred through membrane disruption and not by inhibition of transport proteins since blocking several transport mechanism did not result in decreased leakage [9]. Although not all the toxicological symptoms occurring after glycoalkaloid intoxication of humans and animals can be explained by the proposed mechanism, it might be concluded that sterol mediated membrane disruption is one of the important mechanisms of glycoalkaloid induced intoxication.

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