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SYNERGISTIC INTERACTION BETWEEN THE POTATO GLYCOALKALOIDS α-SOLANINE AND α-CHACONINE IN RELATION TO LYSIS OF PHOSPHOLIPID/STEROL LIPOSOMES

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Key Word Index—*Solanum tuberosum*; *Solanaceae*; liposomes; membrane disruption; steroidal glycoalkaloids; α-solanine; α-chaconine; synergism.

Abstract—At pH 7.2, the steroidal glycoalkaloid α-chaconine disrupted phosphatidylcholine/cholesterol liposomes whereas α-solanine was virtually without effect. A glycoalkaloid mixture extracted from potato sprouts and comprising approximately equal amounts of solanine and chaconine had, at 150 μM, a lytic effect the same as a 150 μM solution of chaconine only. The apparent synergistic interaction between the two compounds was confirmed using 1:1 mixtures of authentic solanine and chaconine from different sources and of different batches. Combinations (1:1) of solanine or chaconine and tomatine or digitonin (both of which lysed liposomes) or β_2-chaconine (which is non-lytic) did not produce synergistic effects. The synergism between solanine and chaconine was observed only when the two compounds were present together, although the order of addition into the test system did not appear crucial. Pretreatment of liposomes with one glycoalkaloid and its subsequent removal did not permanently sensitize the membranes to the second glycoalkaloid. The magnitude of the synergism was dependent on the relative amounts of solanine and chaconine with maximal effects where chaconine comprised 40% or more of the mixture.

INTRODUCTION

Steroidal glycoalkaloids are common in species of *Solanum* and *Lycopersicon* [1] and there is evidence that, because of their toxicity and bitterness [2] they play some role as antimicrobial and/or antifeedant compounds [3, 4]. In lower organisms, these biological effects have their origins largely in the ability of glycoalkaloids to disrupt strategic membranes [4].

In a previous communication [5], we reported that the two major potato glycoalkaloids, α-solanine and α-chaconine, have a markedly different effect on phosphatidylcholine/cholesterol liposomes at pH 7.2, the former having little or no effect at concentrations up to 1 mM but the latter causing extensive membrane disruption and leakage of entrapped contents at 100 μM or less. This finding is consistent with a number of reports [6–11] showing that solanine is also less disruptive than chaconine against cells and organisms. In potato, solanine constitutes a variable but significant proportion of the total glycoalkaloid [12] but it remains unclear why considerable reserves of energy and carbon are channelled into the synthesis of this compound which, although similar to chaconine chemically, is less effective biologically. One possible explanation is that the lytic capacity of each of the two glycoalkaloids is influenced or moderated by the other so that their overall effect *in situ* derives from an interaction between the two compounds. This hypothesis has been tested by examining the effects of solanine and chaconine, both singly and in combination, on the integrity of liposome membranes.

RESULTS

The possibility of an interaction between solanine and chaconine arose from an experiment comparing the effects of these compounds and a glycoalkaloid mixture extracted from potato sprouts on the integrity of phosphatidylcholine/cholesterol liposomes. In agreement with previous findings [5], chaconine was highly active and solanine virtually inactive, but the extracted mixture which contained *ca* 50% of each compound was as effective as a chaconine solution of the same total strength (150 μM). Since this result could be attributed to a maximal effect of chaconine at or below 75 μM, solutions of authentic chaconine were tested at these strengths and also in combination with authentic solanine. The data in Table 1 indicate that chaconine was not maximal in its action at 75 μM and that solanine was inactive at both concentrations. However, when both compounds were present at 75 μM, the disruptive effect was significantly greater than that of 75 μM chaconine and slightly greater than 150 μM chaconine (Table 1). This apparent syner-

Table 1. Effect of solanine and chaconine and their interaction on leakage of peroxidase from liposomes

Treatment	% Peroxidase activity in supernatant
Control	20.6 ± 0.89
Solanine (150 μM)	14.8 ± 0.64
Chaconine (150 μM)	73.2 ± 0.42
Solanine (75 μM)	16.4 ± 0.25
Chaconine (75 μM)	49.7 ± 0.71
Solanine (75 μM) + chaconine (75 μM)	85.6 ± 0.83

Liposomes were treated for 1 hr at pH 7.2. Each value is the mean of five replicate determinations ± s.e.

gism between solanine and chaconine was confirmed on numerous occasions using two different batches of Sigma compounds as well as samples supplied by Dr. S. F. Osman, U.S.D.A., Philadelphia, U.S.A.

To investigate the specificity of this synergism, solanine was tested in combination with α-chaconine, α-tomatine, digitonin and β_2-chaconine. Tomatine and digitonin have been shown to disrupt liposome membranes [13, 14] whereas β_2-chaconine does not [5]. As shown in Fig. 1, a synergistic response occurred only with α-chaconine. In similar interaction experiments using chaconine at both effective (50 μM) and ineffective (25 μM) concentrations

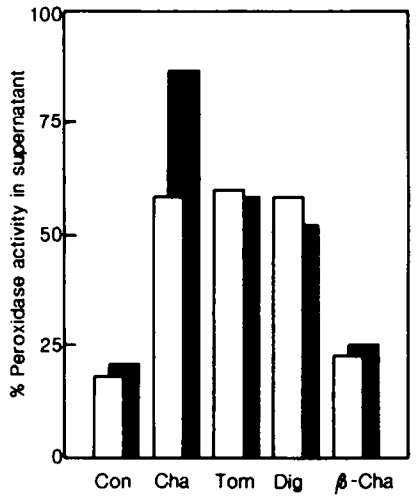

Fig. 1. Interaction between solanine and chaconine, tomatine, digitonin and β_2-chaconine on disruption of liposomes. Open columns represent the single treatments as shown. Con = control; Cha = chaconine; Tom = tomatine; Dig = digitonin; β-Cha = β_2-chaconine: closed columns represent the same treatment but with solanine present. Each compound was present at 75 μM and liposomes were treated for 1 hr at pH 7.2. Digitonin was dispersed in DMSO the final concentration of which in the reaction mixture was 1%. This had no effect on liposomes. Data are means of five replicates. The s.e. range was ±0.36–2.22.

Table 2. Effect of different sequences of solanine and chaconine treatments on disruption of liposomes

Treatment 1	% Peroxidase activity in supernatant	Treatment 2	% Peroxidase activity in supernatant
Control	15.9±0.23	Control	20.6±0.43
Chaconine	41.0±1.31	Solanine	81.1±0.52
Chaconine	40.7±0.42	Chaconine	70.4±0.95
Solanine	17.0±0.60	Chaconine	76.8±0.83

Liposomes were treated with 75 μM alkaloid for 1 hr at pH 7.2 (treatment 1) after which some tubes were removed for peroxidase assay. To others, further additions of 75 μM alkaloid were made and mixtures incubated for a further 1 hr (treatment 2). Each figure is the mean of five replicates ± s.e.

no synergisms with tomatine, digitonin or β_2-chaconine were observed.

Pretreatment of liposomes with one glycoalkaloid followed by treatment with the same or the second glycoalkaloid showed (Table 2) that addition of solanine to a chaconine treatment resulted in a further release of peroxidase, with a final value (81.1%) slightly greater than that from a second addition of chaconine (70.4%). Of interest also was that a chaconine addition to a solanine pretreatment which had no lytic effect in the first hour, elicited in the second hour only a disruptive action (76.8%) of the same order as the chaconine only and the chaconine/solanine treatments, in both of which disruption had taken place over a 2-hr period (Table 2).

Similar two-treatment experiments were also conducted in which liposomes were washed twice between treatments. The effectiveness of the washing procedure in removing the 'first' glycoalkaloid was checked by TLC (silica gel G, 95% EtOH) of MeOH extracts of liposomes. The data in Table 3 indicate that no synergism could be observed in the chaconine/solanine treatment (*cf.* Table 2) when the chaconine was washed out before the solanine addition. Addition of chaconine to a washed solanine treatment resulted in some loss of peroxidase (52.4%) but

Table 3. Effect of different sequences of solanine and chaconine treatments on disruption of liposomes with washing of liposomes between treatments

Treatment 1	% Peroxidase activity in 1st supernatant		Treatment 2	% Peroxidase activity in 2nd supernatant	% Peroxidase activity in 2nd pellet
Control	23.1±0.53	Wash	Control	7.2±0.33	69.7±0.78
Solanine	21.4±0.60		Control	7.7±0.25	70.9±0.69
Chaconine	53.3±0.80		Control	7.1±0.47	39.6±0.82
Chaconine	50.1±1.14		Solanine	7.6±0.24	42.3±1.15
Solanine	16.2±0.26		Chaconine	52.4±0.76	31.4±0.92
Control	16.9±0.45		Chaconine	51.5±0.60	31.6±0.61

Liposomes were treated with 75 μM alkaloid at pH 7.2 for 1 hr (treatment 1) after which they were centrifuged at 50 000 *g* for 30 min. The supernatant was assayed for peroxidase activity and the pellet (intact liposomes) washed 2 × with Tris–HCl buffer pH 7.2, centrifuging as above between washes. The washed pellet was resuspended in Tris buffer and treated with 75 μM alkaloid for a further 1 hr (treatment 2). The sum of % peroxidase activity in the second supernatant and pellet therefore corresponds to the enzyme activity in the first pellet. Each figure is the mean of five replicate determinations ± s.e.

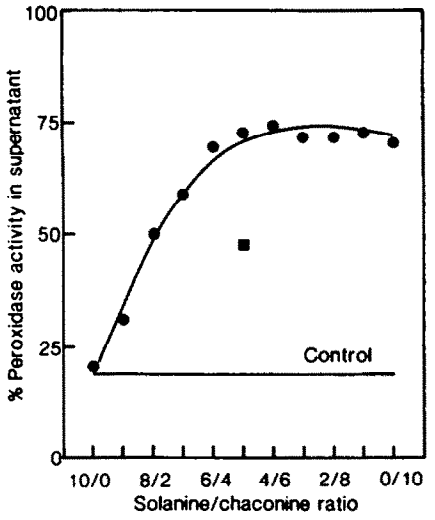

Fig. 2. Effect of different ratios of solanine and chaconine on release of peroxidase from liposomes. Total glycoalkaloid in all non-control solutions was 150 μM distributed between solanine and chaconine as shown. Liposomes were treated for 1 hr at pH 7.2. Each point is the mean of four replicates. ■ represents the effect of 75 μM chaconine only. The s.e. range was ±0.61–2.10.

no greater than that in control tubes subjected to a second treatment of chaconine (51.5%).

Treating liposomes with solanine/chaconine mixtures in ratios other than the 1:1 used above yielded the data shown in Fig. 2. The lack of effect of solanine was again apparent. Increasing the proportion of chaconine up to 40–50% rapidly increased the lytic potential of the mixture but above this level no further disruption occurred. The points on the graph corresponding to zero and 100% chaconine (150 μM) and the additional point inserted for 75 μM chaconine only together confirm that the increase in lysis with increasing proportion of chaconine is a genuine synergistic effect and not a simple dose–response curve for chaconine.

DISCUSSION

The synergism between solanine and chaconine in relation to their membrane-lytic action appears to be a real and potentially important phenomenon. Further, the interaction shows a considerable measure of specificity since other membrane-disruptive steroidal glycoalkaloids (e.g. tomatine) or steroidal saponins (e.g. digitonin) cannot substitute for solanine or chaconine, nor can the non-membrane-disruptive hydrolysis product of α-chaconine, β_2-chaconine. The fact that β_2-chaconine differs from α-chaconine only in lacking one rhamnose molecule points to a key role of the trioside carbohydrate moiety, rather than the aglycone solanidine, in this interaction. Earlier claims [15–17] that the aglycone is the major active moiety of glycoalkaloids have also recently been questioned [5, 13]. The carbohydrate moieties of α-chaconine (chacotriose) and α-solanine (solatriose) are also found in, for example α-solamargine and β-solamarine, and solasonine and α-solamarine, respectively [1] and it would be interesting to know if these glycoalkaloids are able to interact synergistically with solanine and chaconine, respectively. This line of inquiry can be further extended to whether similar interactions occur between other naturally paired glycoalkaloids possessing chacotriose and solatriose, e.g. solasonine and α-solamargine (based on solasodine), α- and β-solamarine (based on tomatidenol). Regrettably, almost no information exists on the biological activity of these (and most other) glycoalkaloids, let alone their interactions. Our findings may even have implications for naturally occurring combinations of other secondary plant metabolites.

It is not yet known whether solanine, virtually inactive in this system, enhances the activity of chaconine or whether chaconine activates solanine. Either way, it is apparent that the synergism requires the simultaneous presence of both compounds (although the order of adding them into the test system is not crucial) and that the liposome membrane is not permanently sensitized to a particular glycoalkaloid by pretreatment with the other. The disruption of liposomes by chaconine is dependent on some association with free sterols in the membrane [5]. Solanine, like chaconine, binds to 3β-hydroxy sterols *in vitro* but often to a lesser extent than chaconine [5]. The possibility therefore exists that the synergism between these two glycoalkaloids has its origins in their sterol-binding capacity.

Fortuitously, the glycoalkaloid mixture used in this study (comprising *ca* 50% of each compound) proved to lie in the range of optimal solanine/chaconine ratios. The fact that in potato tissues chaconine usually comprises at least (and often more) than 50% of the total glycoalkaloid [12] is probably not without significance. The differential effectiveness of different solanine/chaconine combinations and the synergism between the two compounds raises some important questions regarding the evolution of glycoalkaloid patterns as well as about the effects and role of these compounds *in situ*. At this stage however, it would be unwise to extrapolate from liposomes to organisms without knowing whether potato glycoalkaloids interact similarly on the membranes of living cells. Work is currently being done to answer this question and to clarify whether the solanine/chaconine synergism operates at the level of sterol binding.

EXPERIMENTAL

Preparation of liposomes. Cationic liposomes were prepared by a modification of the method of Magee *et al.* [18] with all procedures being carried out at room temperature, unless otherwise stated. Fifty mg of egg yolk phosphatidylcholine (Sigma type VII-E), 10 mg cholesterol and 2 mg stearylamine were dissolved in 20 ml $CHCl_3$–MeOH (9:1) and the soln evapd to dryness under vacuum. The flask was flushed with N_2 and 1.5 ml of a soln containing 10 mg/ml horseradish peroxidase (Sigma type II) and 30 mg/ml bovine serum albumin (Sigma) in 0.2 M phosphate buffer pH 7.2 was added along with six 4 mm diameter glass beads. Agitation using a vortex mixer removed the wall-deposited lipids into a suspension which was sonicated in an ice bath at 26 kHz for six 20 sec periods alternating with 20 sec pauses. The suspension was diluted to 5 ml with 0.05 M Tris–HCl buffer pH 7.2 and centrifuged at 50 000 *g* for 30 min at 4°. The liposome pellet was washed twice in 5 ml Tris buffer, centrifuging as previously between washes, and finally suspended in 1 ml Tris buffer and used immediately.

Sources of test chemicals. Two different batches of solanine and chaconine were purchased from Sigma and samples of these glycoalkaloids and β_2-chaconine were donated by Dr. S. F.

Osman, U.S.D.A., Philadelphia, U.S.A. A sample of β_2-chaconine was also obtained from Dr. G. R. Fenwick and Dr. K. R. Price, AFRC Institute of Food Research, Norwich, U.K. Tomatine and digitonin were purchased from Sigma. A glycoalkaloid mixture was extracted from potato sprouts as described previously [19]. TLC of this mixture (silica gel G) in 95% EtOH and BuOH–HOAc–H_2O (10:3:1) revealed only two major Dragendorff-positive spots which corresponded with authentic solanine and chaconine. The two glycoalkaloids were separated by prep. TLC (silica gel G 0.5 mm, 95% EtOH) and eluted with EtOH. The eluate vols were adjusted to 10 ml and 0.4 ml aliquots removed for assay by a modification of the method of Bergers [20]. Each aliquot was evaporated to dryness and re-dissolved in 0.4 ml 7% (w/w) H_3PO_4 to which was then added 4 ml 70% (w/w) H_2SO_4. After incubating for 10 min at room temp. absorbance was read at 405 nm and referred to a calibration graph prepared with authentic glycoalkaloids. Calibration graphs were linear up to 1 mg/ml. Based on three replicate determinations, the proportions of solanine and chaconine in the glycoalkaloid mixture were deduced to be 47 and 53%, respectively.

Treatment with glycoalkaloids. Glycoalkaloids were prepared as a 2 mM soln by dissolving in 100 mM HCl and diluting the acid also to 2 mM. To 50 μl of liposome suspension were added 3.65 ml Tris–HCl buffer pH 7.2 and 0.3 ml glycoalkaloid (or control) soln. Reaction mixtures were incubated at 25° for 1 hr then centrifuged at 50 000 *g* for 30 min at 4°. The supernatant was decanted and the pellet re-suspended in 4 ml Tris–HCl buffer pH 7.2. The extent of liposome disruption was assessed by determining peroxidase activity in the pellet and supernatant.

Peroxidase assay. The assay was based on oxidation of pyrogallol by oxygen liberated from peroxidase-treated H_2O_2. Peroxidase activity in the supernatant was determined by transferring 0.1 ml to a 10 mm spectrophotometer cuvette followed by 2.5 ml 25 mM pyrogallol in 10 mM phosphate buffer pH 7.0, 0.225 ml distilled water and finally 0.25 ml 1% H_2O_2. Pyrogallol and H_2O_2 solns were freshly prepared and the former protected from light. After mixing, the absorbance of the reaction mixture at 420 nm was immediately recorded on a chart recorder operating at 20 sec/cm and the rate of change of absorbance (ΔA_{405}/sec) used as a measure of peroxidase activity. The peroxidase activity in liposome pellets was determined as above except that 0.025% Triton X-100 was used instead of water to disrupt the liposome membranes. All experiments were carried out at least two times with individual treatments comprising between three and five replicates as indicated.

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