Development and Characterization of Monoclonal Antibodies That Differentiate between Potato and Tomato Glycoalkaloids and Aglycons[†]

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A series of monoclonal antibodies (Mabs) that bind the potentially toxic glycoalkaloids and the corresponding aglycons found in potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) has been developed. Most of these Mabs differentially bind solanidine versus the potato glycosides α-solanine and α-chaconine. Some bind solanidine, α-solanine, and α-chaconine with nearly equal affinity. Others bind only solanidine, and one binds the tomato glycoside α-tomatine and the corresponding aglycon, tomatidine. Fifty percent inhibition of control values in a competition ELISA ranged from 2.5 to 1000 ppb. Specificity and cross-reactivity are discussed in relation to three-dimensional, computer-generated molecular models of selected alkaloids. Results of analyses of potato samples by both the ELISA and an HPLC method are given and discussed. The data suggest that these antibodies provide a basis to develop sensitive, rapid, and inexpensive immunoassays for these alkaloids in plants, processed foods, and body fluids and tissues.

Keywords: *Antibodies; chaconine; ELISA; HPLC; immunoassays; molecular models; monoclonal antibodies; potato glycoalkaloids; solanidine; solanine; solasodine; tomatidine; tomatine; tomato glycoalkaloids*

INTRODUCTION

Alkaloids are potentially toxic nitrogen-containing secondary plant metabolites found in numerous plant species, including potatoes and tomatoes (Friedman, 1992). The two major glycoalkaloids in commercial potatoes (*Solanum tuberosum*), α-chaconine and α-solanine, are both glycosylated derivatives of the aglycon solanidine. Wild potatoes (*Solanum chacoense*) and eggplants (*Solanum melongena*) produce the glycoalkaloid solasonine. The major glycoalkaloid in tomatoes (*Lycopersicon esculentum*), α-tomatine, is the glycosylated derivative of the aglycon tomatidine. Figure 1 illustrates the structures of the potato and tomato alkaloids evaluated in this study.

Methodologies for the analysis of glycoalkaloids and related compounds include gas chromatography (GC) (Herb et al., 1975; Lawson et al., 1992; Van Gelder et al., 1989), high-performance liquid chromatography (HPLC) (Bushway, 1982; Bushway et al., 1979, 1986; Carman et al., 1986; Friedman and Levin, 1992; Morris and Lee, 1981; Osman and Sinden, 1989), and immunoassays based on polyclonal antibodies (Plhak and Sporns, 1992; Ward et al., 1988). No single method has gained widespread acceptance, but HPLC is increasingly being used to analyze individual alkaloids.

Detection of alkaloids in potato and tomato plants is of interest because of the toxic nature of these compounds. Traditional methods to detect these plant constituents are often complicated and time-consuming, and they rely upon the use of large amounts of organic solvents. In addition, the reported methods are not readily field-portable. Most require sophisticated and expensive equipment and trained personnel to run the assays and interpret the results. Clearly, simpler alternative methods are desirable. Immunochemical assays are rapid and simple in design and do not require expensive instrumentation.

These considerations prompted us to develop a series of monoclonal antibodies that bind potato and tomato glycoalkaloids and the corresponding aglycons, which lack the carbohydrate side chains.

Since the structures of the potato and tomato alkaloids are similar from the viewpoint of an antibody, it was of interest to determine whether monoclonal antibodies induced by one structure would recognize others and whether a panel of antibodies could be isolated that differentiated between the structurally related glycoalkaloids and the corresponding aglycons. The ultimate objective of our studies is to devise sensitive, rapid, and low-cost enzyme-linked immunosorbent assays (ELISAs) to measure the *Solanum* alkaloid content in various potato and tomato cultivars, foods, and feeds and in body tissues and fluids. Before this can be achieved, however, a need exists to define the stereochemical and structural features of *Solanum* alkaloids that favor antibody binding. Three-dimensional molecular models of selected alkaloids were evaluated in an effort to better explain the observed antibody cross-reactivity. A related objective was to determine whether these monoclonal antibodies could be used to measure the specific glycoalkaloid content of potato extracts.

MATERIALS AND METHODS

Reagents. The following chemicals were obtained from Sigma Chemical Co., St. Louis, MO: α-chaconine, solanidine, α-solanine, solasodine, tomatidine, and α-tomatine. Choles-

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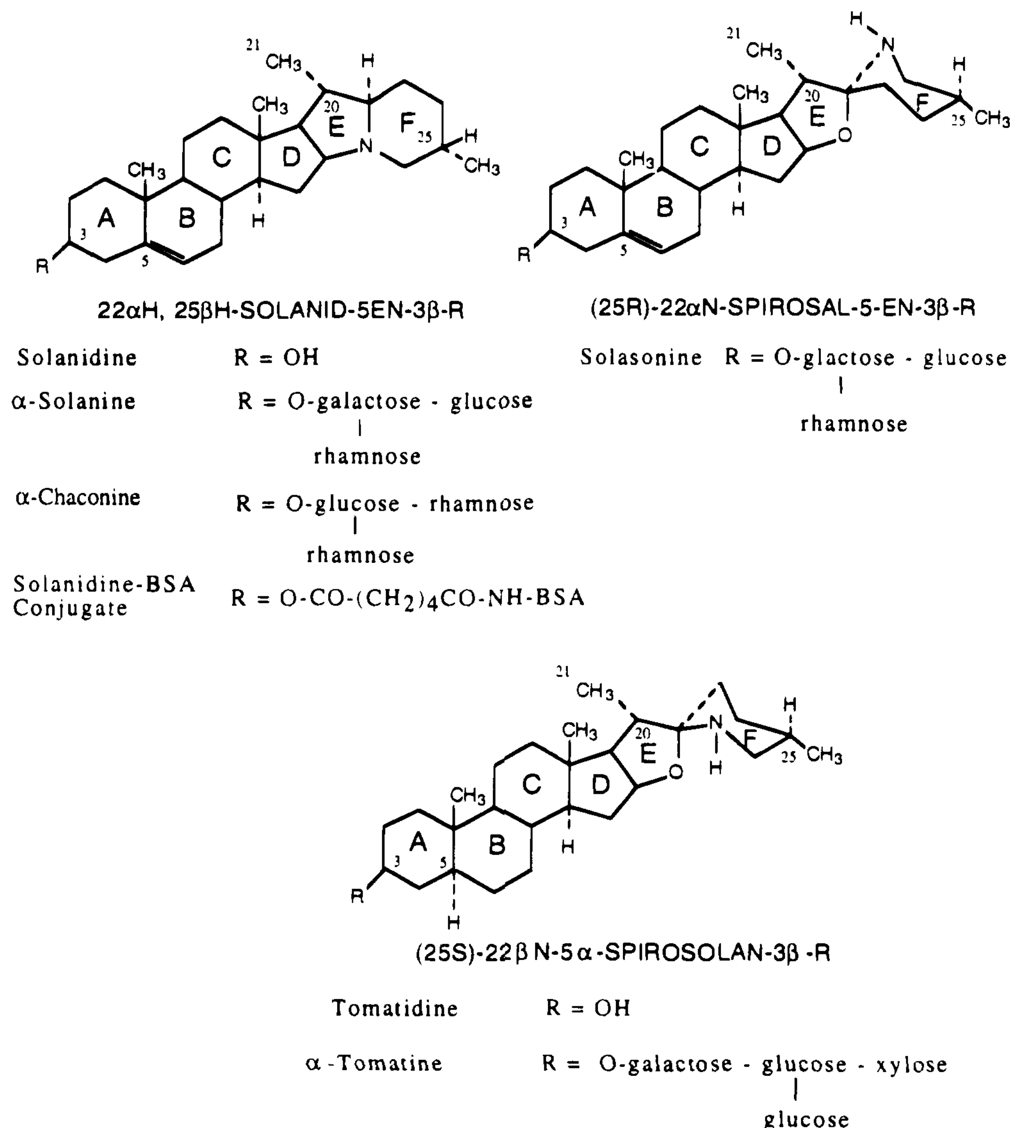

Figure 1. Structures of potato and tomato alkaloids evaluated in this study.

terol, digitonin, β-sitosterol, and stigmasterol were obtained from Aldrich Chemical Co., Milwaukee, WI. Solasonine was obtained from Biosynth A.G., Zurich, Switzerland.

Hapten Preparation. The immunogen was prepared as follows. Solanidine (50 mg) was mixed with succinic anhydride (37.5 mg) in anhydrous pyridine (750 μL) and refluxed overnight to form the hemisuccinate. The solanidine hemisuccinate was purified by thin-layer chromatography (TLC) on silica gel 60 F254 plates using a chloroform–methanol (75:25 v/v) solvent system and then conjugated to bovine serum albumin (BSA) and to keyhole limpet hemocyanin (KLH) as follows. The KLH and BSA were dissolved in 25% dimethylformamide (DMF) (5 mg/mL for the KLH and 10 mg/mL for the BSA). One hundred microliters of solanidine hemisuccinate (7 mg in DMF) was mixed with 50 μL of DMF, 20 μL of N-hydroxysuccinimide (NHS) (3 mg) in DMF–H_2O (50:50 v/v), and 20 μL of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) (3 mg) in DMF–H_2O (50:50 v/v) and incubated at ambient temperature for 60 min. The mixture was then split and 1 mL of the above KLH or BSA solution added. These were then allowed to incubate with stirring at ambient temperature overnight. The samples were dialyzed against deionized water for 3 days, changing the water every 12 h. Conjugation of the hapten to the protein was confirmed by electrophoresis (Kamps-Holtzapple et al., 1993).

Monoclonal Antibody Production. Five, 6-month-old BALB/c mice were injected with 50 μL of the solanidine–KLH conjugate mixed 1:1 with Ribi adjuvant (Ribi Immunochem Research Inc., Hamilton, MT). The mice received half the dose intraperitoneally and half intramuscularly every other week for a total of three injections. One month later, and 4 days prior to fusion, a selected mouse was given an intraperitoneal injection of 50 μg of solanidine–KLH conjugate in sterile saline. The spleen was removed and the splenocytes fused with SP2/0 myeloma cells (Stanker et al., 1986). The fused cells were plated over 30 96-well microculture plates previously coated with mouse peritoneal exudate cells (macrophage) and grown under conditions described by Elissalde et al. (1993).

A direct-binding ELISA, described by Stanker et al. (1993) and modified as described below, was used to screen the culture fluids from the growing hybridomas for antibodies to solanidine. Solanidine linked to BSA (SOL–BSA) served as antigen in both the direct-binding ELISA and the competition ELISA (c-ELISA) (described below). Microtiter plates (Nunc Maxisorb, Roskilde, Denmark; or Costar high binding plates, Costar Corp., Wilmington, MA) were coated with SOL–BSA by addition of 100 μL/well of a 1 μg/mL solution of SOL–BSA in distilled water. The SOL–BSA was incubated in uncovered plates at 37 °C for 18 h to evaporate the liquid and allow the SOL–BSA to coat the bottom of the microtiter wells. The "coated" plates were then stored in sealed plastic bags at 4 °C and used within 2 weeks. Nonreacted sites on the plastic microtiter plates were blocked by adding 400 μL of a 3% solution of nonfat dry milk prepared in assay buffer (0.1 M Tris, 0.15 M NaCl, 10 mg/mL nonfat dry milk, 0.001% Tween 20, 0.02% sodium azide) and then incubating the plates at room temperature for 1 h. Next, the blocking solution was removed, 100 μL of the hybridoma supernatant(s) or anti-

Table 1. Cross-Reactivity of the Different Monoclonal Antibodies: IC_{50} Values[a]

compd	antibody: isotype:	Sol-8 IgG2a	Sol-48 IgG2a	Sol-54 IgG2a	Sol-55 IgG2a	Sol-59 IgG2a	Sol-67 IgG1	Sol-68 IgG1	Sol-71 IgG2a	Sol-91 IgG2a	Sol-106 IgG2a	Sol-129 IgG1
solanidine		46 ± 6.4	18 ± 4.4	131 ± 53	60 ± 19	16 ± 1.7	15.6	489 ± 73	1020 ± 500	36 ± 5.7	14 ± 2.4	2.5 ± 0.35
α-solanine		253 ± 64	436 ± 135	215 ± 32	118 ± 18	35 ± 8	27.8	nc	nc	104 ± 18	49 ± 8	2.6 ± 0.25
α-chaconine		294 ± 34	323 ± 125	165 ± 58	204 ± 19	25 ± 2.1	20.2	nc	nc	82 ± 5.2	54 ± 13	2.8 ± 0.2
solasonine		nc[b]	nc	nc	nc	nc	nc	nc	nc	nc	nc	36 ± 3.2
α-tomatine		nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	5.4 ± 3.5
tomatidine		nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	10.4 ± 2.0
digitonin		nc	nd[c]	nc	nc	nc	nc	nc	nc	nc	nc	nc
cholesterol		nc	nd	nc	nc	nc	nc	nc	nc	nc	nc	nc
stigmasterol		nc	nd	nc	nc	nc	nc	nc	nc	nc	nc	nc
β-sitosterol		nc	nd	nc	nc	nc	nc	nc	nc	nc	nc	nc

[a] IC_{50} values in ppb ± one standard deviation. [b] nc, no competition at 10 000 ppb. [c] nd, not done.

solanidine monoclonal antibodies (from ascites fluid) was added, and the plates were incubated at 37 °C for 1 h. The plates were then carefully washed with a solution of 0.05% Tween 20 in water, and peroxidase-conjugated goat anti-mouse antiserum (Sigma), diluted 1:500 in assay buffer, was added to each well. Following a second 1 h incubation at 37 °C, the plates were washed again and the substrate, 2,2′-azinobis-(ethylbenzthiazole-6-sulfonic acid) (ABTS), was added. Absorbance was measured at 405 nm and the resulting data analyzed by computer using an Excel (Microsoft, Redmond, WA) spreadsheet program.

Hybridoma cells from wells showing a positive response in the ELISA screen were expanded and subcloned twice by limiting dilution to ensure their monoclonal origin. Ascites fluid was prepared in pristane primed mice (Stanker et al., 1986), and the monoclonal antibodies were purified from the ascites by affinity chromatography on Protein-G Sepharose. Isotype determination was done by ELISA using mouse heavy- and light-chain-specific antisera (Southern Biotechnology Associates, Birmingham, AL).

Indirect cELISA. cELISA for solanidine and other related glycoalkaloids was carried out as previously described for other compounds (Stanker et al., 1993). Briefly, to each well of an antigen-coated, nonfat-dry-milk-blocked, microtiter plate was added 100 μL of assay buffer. Competitors dissolved in 100 μL of assay buffer were added to the microassay plate and serially diluted (a 2-fold series). Next, 100 μL of assay buffer containing a predetermined amount of anti-solanidine monoclonal antibody was added to each well. The amount of anti-alkaloid antibody added was the concentration resulting in approximately 50% of maximum signal in a direct-binding ELISA where no competitor was present. The plates were sealed with plastic wrap, incubated for 1 h at 37 °C, and then processed as described above.

In each experiment, microtiter wells containing all components except competitor were prepared and the activity in these wells was taken to represent 100% activity (control wells). The test wells, each containing different amounts of competitor, were normalized to the 100% activity wells, and percent inhibition was calculated as

$$\% \text{ inhibition} = [1 - (A_{405} \text{ of test}/A_{405} \text{ of control})] \times 100$$

where A_{405} is the absorbance at 405 nm.

Alkaloid Extraction. The following potato samples were used in these studies: 1, Klamath tuber flesh (no peel); 2, Russet whole tuber; 3, 3194 whole tuber; 4, Z whole tuber; and 5, Lenape peel (Friedman and Dao, 1992). Freeze-dried samples (100 mg) were suspended in 5 mL of 2% acetic acid and extracted using a polytron homogenizer (3 × 30 s pulse). The resulting slurry was centrifuged at 1000*g* for 10 min. The supernatant was recovered and adjusted to pH 6.5–6.8 by addition of 1 M NH_4OH. Between 2 and 10 μL of the extract was added to 100 μL of assay buffer and used in the cELISA described above.

HPLC Method. The α-chaconine and α-solanine contents of potato extracts were determined by HPLC as described previously (Friedman et al., 1993).

Molecular Modeling Studies. *Determination of Minimum Energy Conformations.* Molecular modeling studies were performed using a CAChe WorkSystem running on a Macintosh Quadra 700 equipped with an RP88 coprocessor board and a CAChe stereoscopic display (CAChe Scientific, Inc.; Beaverton, OR) as previously described (Carlin et al., 1994). Minimum energy conformations were calculated using Allinger's standard MM2 force field (Allinger, 1977) augmented to contain force field parameters for cases not addressed by MM2 (CAChe Scientific). Following the initial optimization, a sequential search for low-energy conformations was performed by rotating all dihedral angles (except for those where the stereochemistry is known) through 360° in 60° increments. The structures resulting from all computations were viewed and superimposed using the CAChe Visualizer+ application.

Determination of Electronic Properties. The electronic wave function was calculated by solving the Schrödinger equation using the extended Hückel approximation (Hoffmann, 1963). The wave function data were converted into 3D coordinates for visualizing electron densities and electrostatic potentials using the CAChe Tabulator application. The electron probability density value was set at 0.01 electron/Å^3 for all calculations. Electrostatic potentials were calculated in reference to an incoming positive charge and represent repulsive energies.

RESULTS

Hybridoma Production. Spleen cells from a BALB/c mouse immunized with solanidine–KLH were fused with SP 2/0 myeloma cells, and the resulting hybridomas were cultured in 30 96-well microculture dishes. At 11 days after fusion, supernatants from each microculture well were evaluated for antibodies reactive to solanidine–BSA in an ELISA (BSA is an extraneous protein because the immunogen was solanidine–KLH). Therefore, anti-KLH antibodies were not detected. Approximately 130 wells secreted antibodies that recognized the solanidine–BSA conjugate. Cells from these wells were expanded and evaluated for their ability to recognize unconjugated solanidine in a cELISA. From these, 11 stable hybridomas were subcloned, and the isotype of each was determined. These 11 monoclonal antibodies, named with a Sol- prefix followed by the number of the well they were isolated from as shown in Table 1, were found to have either IgG1 or IgG2 heavy chains, and all had kappa light chains.

Monoclonal Antibody Characterization. The 50% inhibition of control (IC_{50}) for the 11 antibodies isolated ranged from 2.5 to 1000 ppb. Indirect competition ELISA curves obtained with monoclonal antibodies Sol-8, Sol-129, and Sol-59 are shown in Figure 2. Similar cELISA curves were generated for each monoclonal antibody, and the IC_{50} for each is shown in Table 1. Sol-129 had the lowest relative affinity for solanidine, with an IC_{50} of 2.5 ± 0.3 ppb, followed by Sol-106, Sol-59, Sol-48, Sol-91, Sol-8, Sol-55, Sol-54, and Sol-71, with

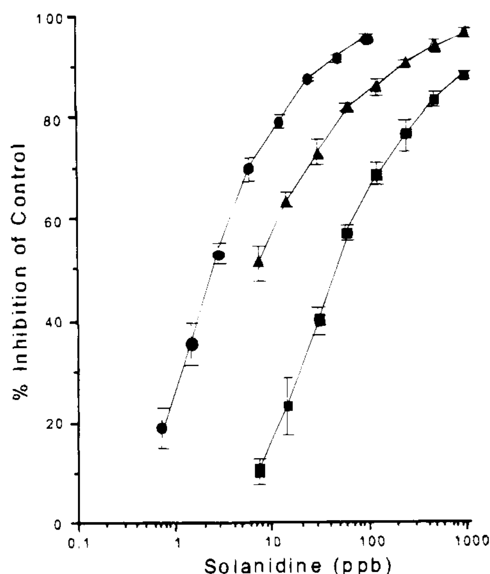

Figure 2. Curves obtained with Sol-8 (■), Sol-59 (▲), and Sol-129 (●) using solanidine as competitor at the concentrations indicated.

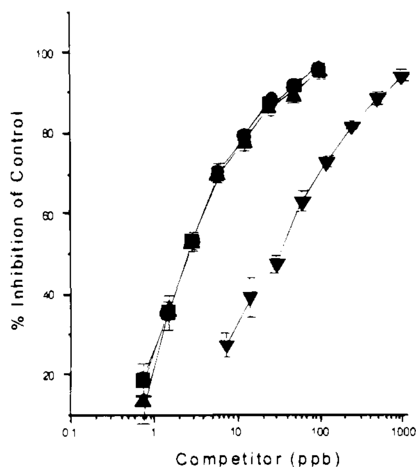

Figure 3. Indirect competition ELISA curves for monoclonal antibody Sol-129 using solanidine (■), α-solanine (●), α-chaconine (▲), and solasonine (▼) as competitors.

IC_{50} values of 15 ± 1.6, 16 ± 1.7, 18 ± 4.4, 36 ± 5.7, 46 ± 6.4, 60.7 ± 19, 131 ± 6.3, and 1020 ± 500 ppb, respectively. Each monoclonal antibody was evaluated further for its ability to bind different alkaloids. Representative cELISA curves are shown in Figures 3 and 4 for Sol-129 and Sol-68 using selected alkaloids as competitor. In some cases (e.g., Sol-106), the antibodies do not differentiate between solanidine and α-solanine and α-chaconine. In others (e.g., Sol-68, Figure 4), the antibodies can differentiate between solanidine and and α-solanine and α-chaconine. The IC_{50} values obtained from these experiments are summarized in Table 1.

Detection in Potato Samples. Five potato samples were analyzed for α-chaconine and α-solanine using an HPLC method. These same samples were analyzed by cELISA using monoclonal antibody Sol-106 following an acid extraction of lyophilized potato samples. The data from these experiments are summarized in Table 2.

Molecular Modeling Studies. *Optimization of Structures.* Computer modeling studies of the structural and electronic properties of the compounds shown in Figure 1 were performed to correlate those properties with the abilities of the compounds to inhibit binding of the anti-solanidine Mabs to solanidine–BSA. The

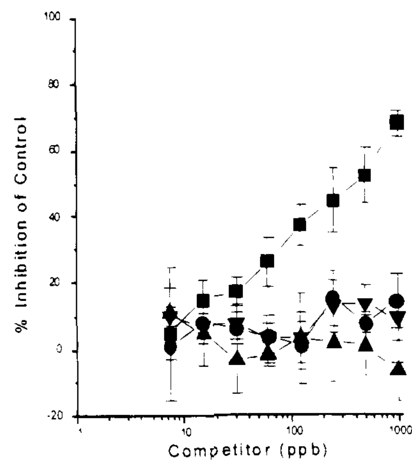

Figure 4. Indirect competition ELISA curves for monoclonal antibody Sol-68 using solanidine (■), α-solanine (●), α-chaconine (▲), and solasonine (▼) as competitors.

Table 2. Glycoalkaloid Levels (Milligrams per Gram of Fresh Weight) Measured in Potato Samples

sample	HPLC α-chaconine	HPLC α-solanine	ELISA total
1	tr	0	0.015
2	0.1	0.1	0.13
3	0.5	0.3	0.5
4	0.8	0.5	0.6
5	2.1	0.8	>2.0

modeled conformations of solanidine, α-solanine, and solasodine are shown in Figure 5 (panels A–C) following rotation of all dihedral angles (except those with a known stereochemical orientation) through 360° and optimization using MM2 force field parameters. Shown in panels A and B is α-solanine (red) superimposed (at the double bond in ring B) on solanidine (blue). The view in panel A demonstrates that the presence of the sugar moieties does not significantly alter the general stereochemistry of the aglycon portion of the molecule. However, rotation of the molecules (panel B) demonstrates that while the overall structures of the solanidine and the aglycon portion of the α-solanine are similar, there is a change in the stereochemistry such that the distances between the molecules in ring A and ring F are greater in solasodine than in α-solanine. A similar situation was observed when α-chaconine was superimposed on solasodine (data not shown). Superimposition of solanidine (blue) and solasodine (yellow) (again at the double bond in ring B) (Figure 5C) demonstrates numerous differences in the stereochemistry, particularly in rings D–F.

Determination of Electronic Properties. In Figure 5, panels D–F show the electrostatic potential calculations displayed on the electron density surfaces for solanidine, α-solanine, and solasidine. The energies are color coded as shown in the figure legend. As can be seen in Figure 5, the aglycon portions of α-solanine and solanidine are almost identical with respect to surface charge. The surface charge of solasodine (panel F) is similar to that of solanidine, particularly in rings A–C. However, a negative region corresponding to an electronegative oxygen atom in ring E (panel F, arrow) is present as well as a slightly more negative region (panel F, arrow head) corresponding to the ring nitrogen.

DISCUSSION

Eleven monoclonal antibodies were isolated following immunization with a solanidine–protein conjugate and

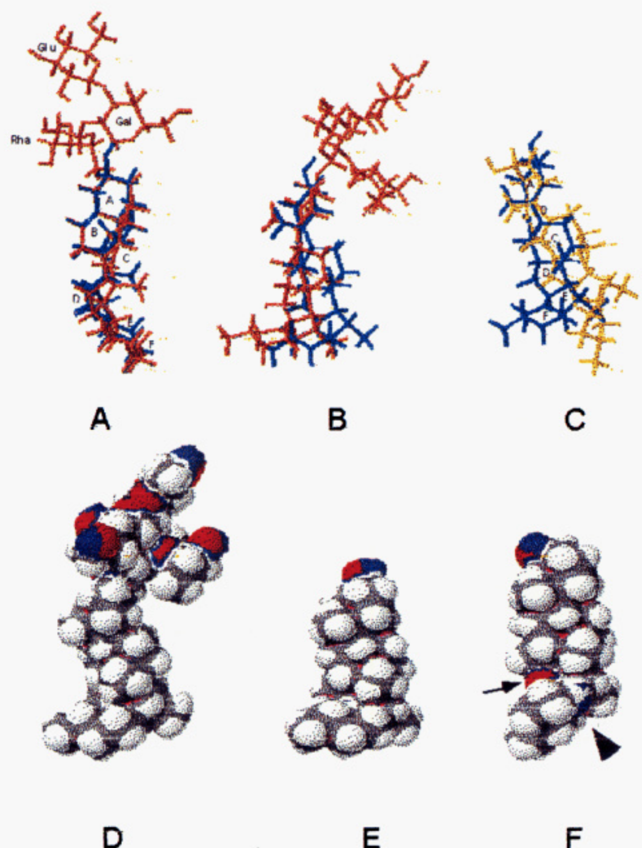

Figure 5. (Top row) A, B, Solanidine (blue) superimposed on α-solanine (red), two different views. C, Solasonine (the aglycon portion) (yellow) superimposed on solanidine (blue). (Bottom row) D–F represent the electron densities of the molecules in A–C (orientation is the same for each molecule). The electron densities are displayed as a simple gradient in increasing order of electronegativity (white < gray < blue < red). The electron probability density value was set at 0.01 electron/Å^3 for all calculations.

cell fusion experiments. Table 1 lists the relative binding affinities of these 11 Mabs to 10 steroidal compounds in terms of 50% inhibition values (IC_{50}). The results show that (a) solanidine is an effective immunogen for generating Mabs; (b) the antibodies show no apparent affinity for steroids lacking ring nitrogen atoms, such as cholesterol, digitonin, β-sitosterol, and stigmasterol; (c) all of the antibodies bound the aglycon solanidine; (d) nine antibodies complexed with the potato glycoalkaloids α-chaconine and α-solanine; (e) only one antibody, Sol-129, had an affinity for solasonine; (f) Sol-129 was the only antibody capable of binding α-tomatine and tomatidine (IC_{50} values of 5.4 and 10.4 ppb, respectively); and (g) Sol-129 clearly had the greatest relative sensitivity for the *Solanum* glycoalkaloids tested.

The data on cross-reactivity patterns of the Mabs with the different alkaloids (summarized in Table 1) and the concentration dependence of the percent inhibition of control binding of the antibodies by solanidine, solasonine, α-chaconine, and α-solanine (shown in Figures 2–4) suggest that it should be possible to select specific antibodies to optimize an immunoassay capable of differentiating structurally related alkaloids. Nearly linear and parallel cELISA curves were observed with most of the Mabs produced. Since antibodies Sol-129 and Sol-106 have nearly identical relative affinities for α-solanine and α-chaconine, they merit further evaluation for their ability to measure alkaloids in a variety of potato plants and processed potato products and in

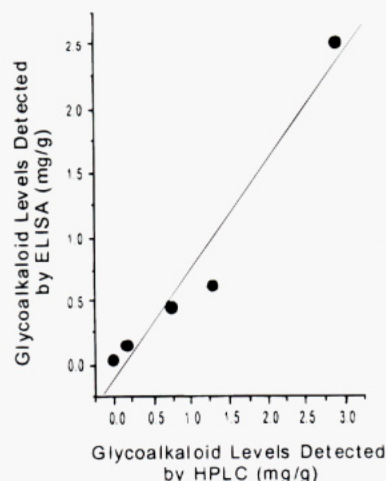

Figure 6. Correlation of glycoalkaloid levels detected in potato samples by HPLC and ELISA. ELISA value = 0.676 × HPLC value − 0.05; $R = 0.9867$.

tissues and fluids of animals and humans. These conclusions are reinforced by our preliminary findings which show that the α-chaconine and α-solanine contents of five potato extracts determined by ELISA correlate well with those determined by HPLC (Table 2; Figure 6). The ELISA method appears to underestimate the amount of *Solanum* alkaloids, probably due to inefficient extraction of these materials.

To define possible structure–antibody binding relationships, it is also instructive to relate the structural variation in the alkaloids shown in Figure 1 to the corresponding cross-reactivities of the different monoclonal antibodies shown in Table 1. As already mentioned, the four steroids lacking ring nitrogens (cholesterol, digitonin, β-sitosterol, and stigmasterol) do not interact with the monoclonal antibodies elicited by the solanidine–protein conjugate. This was not unexpected since conjugation to the carrier protein was via carbon 3. Thus, we would expect that molecular substitutions distal to ring A, particularly in rings E and F, would greatly influence antibody binding. The two potato glycoalkaloids, α-chaconine and α-solanine, share the same aglycon, solanidine, but differ in the nature of the trisaccharide attached to the hydroxyl group at the 3-position. Table 1 shows that the cross-reactivities for these two glycoalkaloids are nearly identical for many of the antibodies produced (e.g., Sol-129, Sol-106, and Sol-67). Other antibodies differentiated between solanidine and α-solanine and α-chaconine (e.g., Sol-71 and Sol-48). The monoclonal antibodies isolated can be divided into four epitope groups on the basis of this reactivity. Group one binds only solanidine, α-solanine, and α-chaconine but distinguishes these glycoalkaloids from the aglycon (solanidine). This is the largest group and includes Sol-8, Sol-48, Sol-55, Sol-91, and Sol-106. The second group binds solanidine, α-solanine, and α-chaconine with roughly equal relative affinities and is represented by Mabs Sol-59 and Sol-67. Group three binds only solanidine and includes Sol-68 and Sol-71. Group four is the rarest, containing only a single antibody, Sol-129. Antibody in this last group binds solanidine, α-solanine, and α-chaconine as well as solasonine, tomatidine, and α-tomatine. These data demonstrate that the sugar moieties can affect the affinity for antibody binding but that in many cases their exact structures do not appear to have a major influence. However, the molecular models presented demonstrate that stereochemical differences in the

aglycon portion of the molecule do exist between solanidine and α-solanine. Changes in the spacing of atoms that are contact points for the antibody clearly could affect antibody binding. One conclusion supported by the cross-reactivity and modeling data is that the binding of Mab Sol-129 to solasonine is probably due to contacts with the B–E rings of solasonine. Also, the higher relative affinity of Sol-129 may be the result of more contact with the hapten than is present in the other antibodies. However, the exact nature of these relationships is unknown.

The data presented here clearly demonstrate that a diverse immune response is observed following simple immunization with an aglycon conjugated to a carrier protein. Thus, a polyclonal antiserum will contain a mixture of antibodies reflecting the diverse immune response of the mouse and will include antibodies that can bind the aglycon independent of the sugar moieties, as well as antibodies sensitive to the sugar moieties.

The glycoalkaloid solasonine, found in a number of potato cultivars and in eggplants, contains an oxygen in ring E, has a spiroketal structure with a methyl group in the α-position in ring F, belongs to the 25*R* stereochemical series, and contains the same sugar moiety, solatriose, as does α-solanine. Only one antibody, Sol-129, bound solasonine. This latter observation further supports the conclusion that for the majority of Mabs described here binding is the result of contacts in rings E and F and is only marginally influenced by the sugar moieties.

The tomato glycoalkaloid, α-tomatine, has a tetrasaccharide attached to the 3-hydroxy group of the aglycon tomatidine. Structurally it is a spirosolane belonging to the 25*S* series, with a reverse *S* configuration at the spiroatom 22 as compared to the spirosolane glycoalkaloid solasonine discussed above. These changes are apparently sufficient to inhibit binding of most of the Mabs isolated except for Mab Sol-129.

Earlier studies by Ward et al. (1988) and Plhak and Sporns (1992) described polyclonal antisera raised using immunogens produced by first cleaving the carbohydrate side chain by periodate to aldehyde groups, followed by Schiff base formation with BSA and reduction of the Schiff bases by sodium borohydride. These conjugation strategies are more complex and may lead to formation of linkages at a variety of positions. Such an immunogen may lead to undesirable cross-reactivities. Our findings suggest that a simple conjugation strategy using the aglycon can be utilized to produce antibodies capable of binding related glycoalkaloids. Furthermore, the monoclonal antibodies described here clearly are capable of detecting these alkaloids in potato and should form the basis of a useful test for measuring the levels of these alkaloids in potato and tomato cultivars.

Our findings on the cross-reactivities of different monoclonal antibodies with structurally different glycoalkaloids and aglycons provide a basis for the utilization of these antibodies for specific analytical needs. For example, a major need is the development of an ELISA assay kit to measure potato and tomato glycoalkaloid content in thousands of plant cultivars produced in breeding programs designed to develop improved varieties. The described method may also find application in the analysis of glycoalkaloids and biosynthetic intermediates in foods and in the elucidation of the distribution of the glycoalkaloids in animal tissues and fluids. Finally, since foods and body tissues may contain both potato and tomato glycoalkaloids and metabolites, a need exists for ELISAs to measure both specific and total glycoalkaloid contents. Studies in progress are aimed at addressing some of these needs.

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