A Capillary Electrophoresis Laser-Induced Fluorescence Method for Analysis of Potato Glycoalkaloids Based on a Solution-Phase Immunoassay. 2. Performance Evaluation

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Glycoalkaloids (GAs) occur naturally in potatoes and are toxic to humans and animals. The objective of the present study was to evaluate the performance of a solution-phase immunoassay coupled to capillary electrophoresis with laser-induced fluorescence (CE−LIF) detection for the determination of total glycoalkaloids in potatoes. The immunoassay was based on a competition between potato glycoalkaloids and fluorescently labeled solanidine. Reaction products were separated in the capillary zone electrophoresis mode. A calibration curve of signal vs log[GA] was linear from 50 to 400 nM. The CV for duplicate and day-to-day analyses averaged 5.7% and 12%, respectively. Spike recoveries ranged from 85 to 97% for spike levels ranging from 43 to 170 μg/g fresh potato. Potato samples with GA concentrations ranging from <40 to >200 μg/g were successfully analyzed, indicating that immuno−CE−LIF is a rapid alternative to traditional ELISA and HPLC methods.

Keywords: *Chaconine; solanine; tuber; competitive immunoassay*

INTRODUCTION

The potato is an important staple crop with global production totaling 293 million tons in 1998 (FAO). As do other members of the *Solanaceae* family, potatoes synthesize steroidal glycoalkaloids (GAs). Several studies have suggested that these alkaloids have evolved to confer pest resistance within the potato plant. Correlations have been found between foliar GA levels and resistance to Colorado potato beetle (Sinden et al., 1980) and potato leafhopper (Tingey et al., 1978), although the type of GA appears to be more important than the total amount of GA. The fact that GA levels rise when a tuber is stressed or injured also implies a protective role.

The potential toxicity of potatoes has long been suspected (Harris and Cockburn, 1918). Because potatoes are so widely consumed throughout the world, elevated GA levels can have significant consequences. Hall (1992) claimed that cyanogenic glycosides and GA are responsible for far more human illness and death than any other plant toxicants. Suspected cases of potato poisoning have been reviewed by Morris and Lee (1984). There are two main mechanisms of toxicity. It is clear that GAs disrupt cell membranes (Gee et al., 1996; Keukens et al., 1996). Cell lysis in the gastrointestinal tract can lead to abdominal cramps, diarrhea, and eventually internal hemorrhaging. Potato GAs are also known to inhibit cholinesterase (Nigg et al., 1996; Schwarz et al., 1995). Cholinesterase enzymes are a key component of the nervous system. The physiological effects of cholinesterase inhibition include muscle spasms, followed by complete loss of function because of continuous depolarization of the cell membrane. The teratogenic effects of potato GA are still being debated (Friedman and McDonald, 1997). From a dietary point of view, there is growing consensus that toxic effects can be seen at consumption levels in the range of 1 to 2 mg/kg body weight (Friedman and McDonald, 1997). Traditionally, it has been accepted that tuber GA levels should be lower than 200 mg/kg (fresh weight); however, there have been suggestions that even this limit is too high (Hellenäs, 1994; Morris and Lee, 1984). Toxicity is also a concern with regard to the byproducts of potato processing such as potato protein concentrate which can have much higher GA levels than tubers (Driedger and Sporns, 1999).

Widespread testing of potatoes for GAs has not been common practice, in part, because traditional chromatographic analyses are very time-consuming. The development of ELISA methodologies has considerably reduced analysis time (Plhak and Sporns, 1992; Stanker et al., 1994). As an alternative to ELISAs, we have investigated the potential of coupling a solution-phase immunoassay with capillary electrophoresis (CE) laser-induced fluorescence (LIF) detection for the rapid determination of GAs in potatoes (Driedger et al., 2000). In this immuno−CE−LIF procedure, potato GAs and a fluorescently labeled alkaloid are allowed to react with a limited amount of anti-GA serum (Figure 1). The potato GAs and labeled-alkaloid compete for antibodies. When CE−LIF is used to separate the products of the immunoreaction, the area of the labeled alkaloid peak is proportional to the amount of GA in the potato extract. The purpose of the present work was to document the reliability of using this immuno−CE−LIF method for the quantification of GAs in potato tubers. Specificity, range, precision, and accuracy were evaluated.

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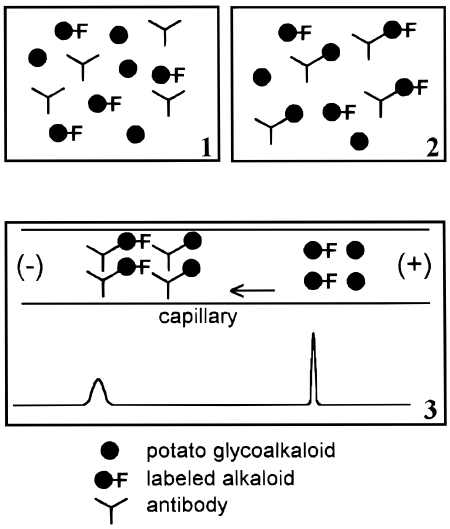

Figure 1. Competitive immunoassay coupled to CE−LIF. (1) Potato GA, labeled-alkaloid, and antibody are mixed, (2) potato GA and labeled-alkaloid compete for antibody binding sites, (3) reaction products are separated by CE−LIF.

MATERIALS AND METHODS

Instrumentation. A Beckman P/ACE System 2100 CE instrument with a laser-induced fluorescence detector was used (Beckman Instruments, Inc., Fullerton, CA). The detector was equipped with a 488-nm laser for excitation and a 520-nm emission filter. Separations were performed on an uncoated fused-silica capillary, with a total length of 27 cm, effective length of 20 cm, and i.d. of 50 μm (Polymicro Technologies, Inc., Phoenix, AZ). Prior to each injection, the capillary was conditioned with 0.1 N NaOH for 1 min, followed by reconditioning with a buffer rinse for 2.5 min. Applied voltage was 10 kV, column temperature was maintained at 25 °C, and sample introduction was by pressure for 2 s. Data were collected with Beckman System Gold software, version 8.10.

Materials. The fluorescent label, 4′(aminomethyl)fluorescein (AMF), was purchased from Molecular Probes, Inc. (Eugene, OR). Other chemicals were obtained from the following suppliers: α-chaconine, solanidine (SOL) and 4-(dimethylamino)pyridine (Sigma Chemical Co., St. Louis, MO); α-solanine (Indofine Chemical Co., Inc., Somerville, NJ); pyridine (Fisher Scientific, Edmonton, AB); succinic anhydride (BDH Inc., Edmonton, AB); *N*-hydroxysuccinimide, 1,3-dicyclohexylcarbodiimide, and *N*,*N*-dimethylformamide (Aldrich Chemical Co., Milwaukee, WI). Polyclonal anti-GA serum (Plhak and Sporns, 1992) was diluted 1:10 in phosphate-buffered saline (PBS) for the immunoassay. PBS solution consisted of 0.9% NaCl and 50 mM phosphate, pH 7.5. Preparation of fluorescently labeled solanidine (AMF−SOL) has been described previously (Driedger et al., 2000). A 600 nM solution of AMF−SOL was prepared in PBS containing 10% methanol (v/v).

Potato (*Solanum tuberosum*) samples A−F were originally described by Abell and Sporns (1996). Samples A and B were cultivar Russet Burbank stored 8 and 12 months, respectively. Samples C and D were cultivars Shepody and Yukon Gold, respectively, both stored 8 months. Samples E and F were obtained commercially. Sample E was peeled and Sample F was unpeeled. A second Yukon Gold potato sample was obtained from Alberta Agriculture, Food and Rural Development, for repeatability studies and had been stored in a commercial seed potato storage facility for approximately 7 months after the 1997 harvest.

GA Extraction from Potatoes. GAs were extracted from freeze-dried potatoes using a modification of a procedure described by Plhak and Sporns (1992). Samples (1.00 g) were initially moistened with 5 mL Milli-Q purified water. The sample was then extracted three times by homogenizing in 15 mL of HPLC-grade methanol for 1 min using a Polytron homogenizer (Kinematica AG, Littau, Switzerland). After each extraction, the sample was centrifuged using a Beckman JA-20 centrifuge (Beckman Coulter Inc., Fullerton, CA) at 1960 × *g* for 5 min. The supernatants from each of the three extractions were then combined and filtered through a Whatman No. 4 filter into a 50-mL volumetric flask. The extract was brought to volume with methanol and stored at 4 °C until it could be analyzed. Prior to analysis, 0.5 mL of the extract was diluted to 50 mL with PBS and methanol so that the final methanol content was 10% (v/v).

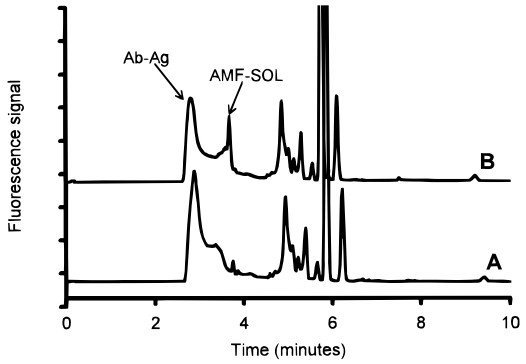

Figure 2. Electropherograms of GA immunoassay products. (A) negative standard and (B) extract of a potato sample containing 53 μg/g GA. Peaks: Ab−Ag, antibody−antigen complex; AMF−SOL, fluorescently labeled solanidine. Experimental conditions: capillary, 50-μm i.d. × 27 cm (20 cm to detector); running buffer, 50 mM phosphate, 1.5 mM SDS, 10% methanol (v/v), pH 7.5; applied voltage, 10 kV; fluorescence excitation at 488 nm and emission at 520 nm.

Glycoalkaloid Standard Curves. A stock solution of 240 μM total GA was prepared in methanol using ca. equal amounts of α-chaconine and α-solanine. Calibration standards were prepared with ca. 0, 50, 100, 200, 300, and 400 nM total GA in PBS with 10% methanol (v/v). Standards were analyzed in duplicate.

Analytical Procedure. Diluted potato extract (250 μL) was mixed with 250 μL of 600 nM AMF−SOL solution and 250 μL of the 1:10 serum dilution. The mixture was allowed to equilibrate for 30 min at room temperature before analysis by CE−LIF. Extracts were assayed in duplicate and each assay injected in duplicate. Peak areas were used to determine total GA concentrations in the extracts.

RESULTS AND DISCUSSION

CE−LIF Analysis of GA Immunoassay Products. Electropherograms of a blank standard and a potato sample containing 53 μg/g GA are presented in Figure 2. A broad antibody−antigen peak eluted approximately 0.6 min prior to the free AMF−SOL. The broad character of the antibody−antigen peak could be due in part to the heterogeneous nature of the polyclonal antibodies used. It could also be due in part to dissociation of the antibody−antigen complex in the capillary. However, the sharp shape of the free AMF−SOL peak and the relatively small degree of peak tailing suggest that dissociation of the antibody−antigen complex does not significantly interfere with the quantification of AMF−SOL. The presence of additional peaks in the electropherogram represent impurities in the original AMF−SOL preparation. These impurities have been shown not to interfere with the immunoassay (Driedger et al., 2000).

Specificity. The specificity of an immunoassay depends on the cross-reactivity of the antibody being used. In the present immuno−CE−LIF method, the area of the unbound AMF−SOL peak will be affected only by compounds that exhibit an affinity for the anti-GA

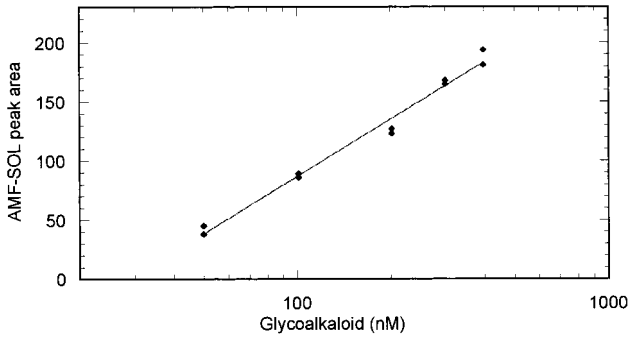

Figure 3. Calibration curve for the determination of potato GAs by CE−LIF−based immunoassay.

serum. The serum used in this experiment was well characterized by Plhak and Sporns (1992). They showed that the serum had a strong affinity for solanidine alkaloids (chaconine and solanine), slightly lower affinity for the structurally similar demissidine alkaloids (demissine and commersonine), and very little affinity for the spiral alkaloids (tomatidine and solasodine). Given that the antibody had little affinity for spiral potato alkaloids, the likelihood that other less-related compounds could interfere with the method is remote. The strong affinity for solanidine alkaloids makes the antibody suitable for most potato analyses since chaconine and solanine make up at least 95% of the GAs found in domestic potato varieties (Friedman and McDonald, 1997). However, this procedure may underestimate total GA levels in nondomesticated potato varieties, which can contain a variety of nonsolanidine alkaloids (Maga, 1980).

Calibration and Range. A new calibration curve was generated each day the CE−LIF analysis was performed. When plotted on a semilogarithmic scale, a straight-line calibration curve was generated with standards encompassing 50−400 nM GA (Figure 3). Extension of the range beyond these values produced a sigmoidal curve typical of competitive immunoassays (Schmalzing et al., 1995a). For quantification, the range was limited to the straight-line portion of the curve. The latter allowed for quantification of GA in tubers from 43 to 340 μg/g on a fresh weight basis under the specified protocol for sample preparation. Samples E and F contained less than 40 μg/g GA and were reported as having trace amounts of GA. It is expected that levels less than 40 μg/g could be quantified by CE−LIF by using more concentrated sample extracts, but because the generally recognized acceptable concentration of GA in tubers is 200 μg/g, no further effort was made to accurately quantify the samples containing less than 40 μg/g GA.

Accuracy. Without standard reference material, it was impossible to conclusively determine the accuracy of the method. Nevertheless, the accuracy was estimated with spike recoveries and by comparing results with different analytical methods. Spike recoveries ranged from 63% to 97% with the lowest recovery coming from the lowest spike level (Table 1). Similar recoveries were reported by Schmalzing (1995a,b). Data from the same potato samples analyzed by HPLC and matrix-assisted laser desorption/ionization mass spectrometry (MALDI−MS) are presented in Table 2. While each method successfully identified high and low GA samples, there were differences in actual values. HPLC values tended to be somewhat higher than CE−LIF and MALDI−MS values. However, if CE−LIF values are adjusted for spike recoveries (typically 90%), agreement between CE−LIF and HPLC data improves. Different extraction procedures no doubt account for some of the discrepancies; freeze-dried HPLC samples were extracted with methanol, whereas freeze-dried MALDI−MS samples were extracted with methanol/water, 1:1 (v/v) (Abell and Sporns, 1996). Nevertheless, it should be noted that previous comparisons of GA analytical methods exhibited similar agreement between immunoassay and HPLC data as was observed in our experiment (Plhak and Sporns, 1992; Hellenäs, 1984). Recently, somewhat better agreement between ELISA and HPLC was reported by Friedman et al. (1998).

Table 1. Accuracy of Potato GA Determination[a]

GA added (μg/g)	GA found (μg/g)	recovery (%)
22	14	63
43	41	97
86	73	85
170	160	93

[a] Initial concentration in potato sample was 43 μg/g.

Table 2. Comparison of GA Analysis by CE−LIF, HPLC, and MALDI−MS[a]

	total glycoalkaloids (μg/g, fresh weight basis)		
sample	CE−LIF	HPLC	MALDI−MS
A	210	252	217
B	220	340	364
C	97	131	92
D	98	107	76
E	trace	12	16
F	trace	22	29

[a] HPLC and MALDI−MS data published by Abell and Sporns (1996).

Repeatability. The immunoassay and separation by CE−LIF were performed on duplicate aliquots from the same extract. The coefficients of variation (CV) for the duplicates ranged from 0.9% to 9.7% with an average of 5.7% ($n = 14$). To determine the variability due to the instrument, a 200-nM AMF−SOL in PBS solution was analyzed as an instrument check sample three or four times with each sample queue. On a given day, the CV for peak areas for the instrument check sample averaged 5%, indicating that the instrument accounted for a large proportion of the intra-assay variability. This suggests that the use of an internal standard may be helpful in clarifying some of the variability due to the instrument. The day-to-day repeatability of the method was estimated by analyzing a Yukon Gold potato extract on four separate days. A mean value of 53 μg/g was obtained with a CV of 12%. The repeatability of the present CE−LIF method is comparable to other solution-based immunoassays (Schultz et al., 1995; Schmalzing et al., 1995b).

These results indicate that a solution-phase immunoassay coupled to CE−LIF allows for rapid quantification of total GAs in potatoes. The sample extract was incubated with the antibody solution for 30 min and was followed by a 10 min CE−LIF separation. Shorter incubation times are likely possible, but were not investigated. Because CE−LIF injection is automated, the analyst simply has to mix the reagents in a microcentrifuge tube and allow them to incubate before transferring the contents to a CE vial. By adjusting volumes, it is likely the whole immunoassay could be performed in the CE vial itself. The short analysis time and potential for fully automated operation make this technique attractive for routine analysis of large num-

bers of samples. At the same time, the simplicity of the method makes it convenient for the analysis of small numbers of potato GA samples as well.

ABBREVIATIONS USED

AMF, 4′(aminomethyl)fluorescein; CE, capillary electrophoresis; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; GA, glycoalkaloid; MALDI−MS, matrix-assisted laser desorption/ionization−mass spectrometry; PBS, phosphate-buffered saline solution; SOL, solanidine.

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