The Primary *In Vivo* Steroidal Alkaloid Glucosyltransferase From Potato

Kent F. McCue[a,*], Paul V. Allen[a], Louise V.T. Shepherd[b], Alison Blake[b], Jonathan Whitworth[c], M. Malendia Maccree[a], David R. Rockhold[a], Derek Stewart[b], Howard V. Davies[b], & William R. Belknap[a]

[a]USDA, Agricultural Research Service, Crop Improvement and Utilization Research Unit, 800 Buchanan St., Albany, CA 94710 USA
[b]Scottish Crop Research Institute, Quality, Health and Nutrition Programme, Dundee DD2 5DA UK.
[c]USDA, Agricultural Research Service, Small Grains & Potato Germplasm Research Unit, 1693 S 2700 W, Aberdeen, ID 83210 USA

Abstract

To assist control of the steroidal glycoalkaloid (SGA) pathway in potato we have investigated the steroidal alkaloid glycosyltransferase (*Sgt*) gene family. The committed step in the SGA pathway is the glycosylation of solanidine by either UDP-glucose or UDP-galactose leading to α-chaconine or α-solanine, respectively. In this study we identify the *Sgt2* gene, and the function of the gene product (SGT2), that serves as the primary UDP-glucose:solanidine glucosyltransferase *in vivo*. The *Sgt2* gene was identified by deduced protein sequence homology to the previously identified *Sgt1* gene. *Sgt1* has glucosyltransferase activity *in vitro*, but *in vivo* serves as the UDP-galactose:solanidine galactosyltransferase. Accumulation of α-solanine was enhanced and α-chaconine was inhibited in the tubers of transgenic potatoes (*Solanum tuberosum*) cvs. Lenape and Desirée expressing an antisense *Sgt2* gene construct. Studies with recombinant SGT2 protein expressed in yeast and purified by metal ion affinity chromatography show that SGT2 glycosylation activity is highly specific for UDP-glucose as a sugar donor.

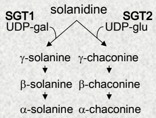

Fig. 1. Glycosylation steps of the SGA biosynthetic pathway. Solanidine is the branch point for synthesis of the two predominant potato glycoalkaloids catalyzed by SGT1, the UDP-galactose:solanidine galactosyltransferase and SGT2 the UDP-glucose:solanidine glucosyltransferase.

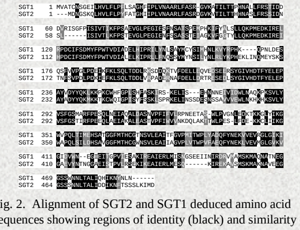

Fig. 2. Alignment of SGT2 and SGT1 deduced amino acid sequences showing regions of identity (black) and similarity (gray).

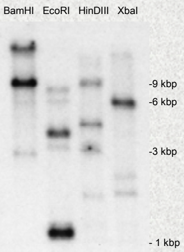

Fig. 3. Occurrence of *Sgt2* in the potato genome. Genomic DNA analysis using 10μg of DNA from *S. tuberosum* cv. Lenape cut with the restriction endonucleases as indicated and probed with the 1,066 bp amino terminal fragment of *Sgt2*.

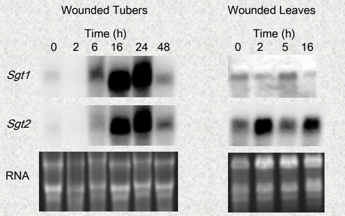

Fig. 4. Expression of *Sgt1* and *Sgt2* RNA in control and wounded potato tubers and leaves. Potato cv. Lenape tubers and leaves were wounded for the times indicated and total RNA (20μg/lane tubers, 10μg/lane leaves) was probed with *Sgt1* and *Sgt2*. The ethidium bromide stained gel is shown to reference RNA loading and integrity.

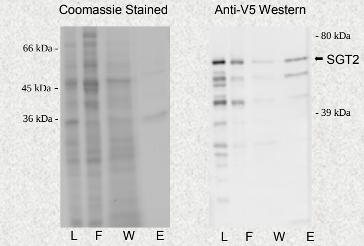

Fig. 5. Recombinant SGT2 protein isolation. Coomassie stained gel and Western blot with anti-V5 antibody The steps in isolation of protein: L-load, F-flow through, W-wash, and E-eluted protein.

Table I. SGT2 UDP-sugar and aglycone preference.

Substrate	UDP-[^{3}H]Glucose (nkat mg^{-1})	UDP-[^{3}H]Galactose (nkat mg^{-1})
Solanidine	1,060 ± 25	NA
Solasodine	880 ± 29	NA
Tomatidine	930 ± 152	NA

Glycosyltransferase activity of the recombinant SGT2:his fusion protein purified from yeast. Values represent the average of duplicate assays ± s.d. (nkat mg^{-1} = moles of product per second per mg protein x 10^{-9}).
NA = No activity, for reactions with dpm counts less than 2-fold the assay blank.

Table II. Effect of UDP-galactose or triose end products, α-solanine or α-chaconine, on recombinant SGT2 glucosyltransferase activity.

Substrate Concentration	UDP-galactose (nkat mg^{-1})	α-solanine (nkat mg^{-1})	α-chaconine (nkat mg^{-1})
33μM	380 ± 7.4	410 ± 102	490 ± 4.8
100μM	500 ± 84	430 ± 33	520 ± 10.6
1,000μM	390 ± 23	250 ± 31	NA

Glucosyltransferase activity of the recombinant SGT2:his fusion protein purified from yeast in the presence of added UDP-galactose or steroidal alkaloid triose end products. Values represent the average of duplicate assays ± s.d.
NA = No activity, for reactions with dpm counts less than 2-fold the assay blank.

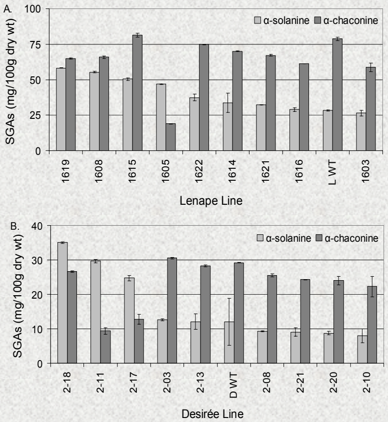

Fig. 6. SGA content of tubers from transgenic potatoes expressing the *Sgt2* antisense transgene. Levels of α-solanine and α-chaconine in selected transgenic and wild type (WT) control lines of A) Lenape and B) Desirée. The plant lines are arranged in the graphs sorted by decreasing α-solanine levels. Values are the average of 3 slices from 3 field-grown tubers (Lenape) or 2 glasshouse-grown minitubers (Desirée). Error bars show s.d.

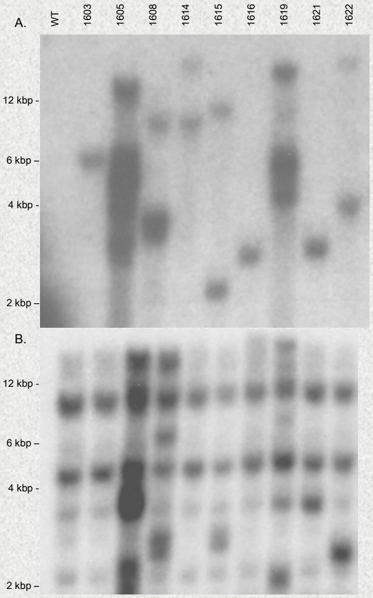

Fig. 7. Integration patterns of antisense *Sgt2* transgene in Lenape lines. Genomic DNA blot analysis of T-DNA insertion in control and transgenic lines of Lenape. Genomic DNA (20 μg/lane) was digested with HinDIII and probed with the A) the complete *NptII* coding sequence, and B) the *Sgt2* amino terminal CDS.

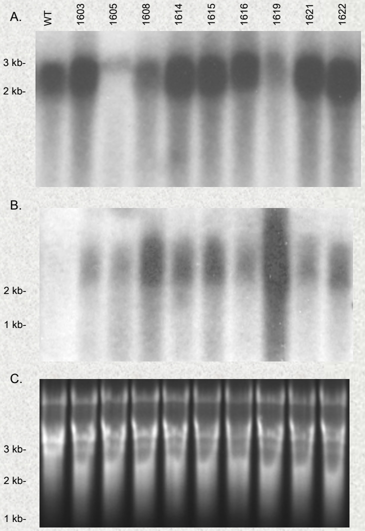

Fig. 8. Steady state RNA expression of *Sgt2* transcripts in Lenape lines. Analysis of steady state levels of total RNA (30μg/lane) in control and transgenic lines of Lenape: A) *Sgt2* messenger levels, B) *NptII* messenger levels, and C) ethidium bromide stained gel.

Conclusions

Two *Sgt2* sequences (*Sgt2.1* and *Sgt2.2*) were isolated from the *S. tuberosum* cDNA library and likely represent two major alleles expressed in the heterozygous tetraploid genome. Extensive homology between the two alleles suggests that both alleles would be simultaneously down-regulated in plants expressing effective antisense genotypes. The genomic DNA blot analysis allows an estimation of gene copy number. The presence of no more than two major bands indicates that *Sgt2* is a low copy gene in *S. tuberosum*. This in combination with 2 distinct coding sequences from PCR and two representative tentative consensus sequences in the TIGR database suggests that there are only two major active alleles.

Potato SGAs are knows to accumulate in tubers upon wounding. As expected the expression of *Sgt1* and *Sgt2* is coordinately regulated in response to wounding in the tubers with nearly identical patterns of mRNA accumulation. Although SGAs are also found in leaves, the pattern of expression is quite different. *Sgt1* is not wound-induced while *Sgt2* shows induction.

The two solanidine glycosyltransferases represent the committed steps for carbon flow into the SGA pathway. Down-regulation of either *Sgt1* or *Sgt2* tends to cause an increase in the accumulation of the end product of the other branch, α-chaconine or α-solanine, respectively. SGT2 is specific for UDP-glucose as the sugar donor. UDP-galactose at concentrations from 33 to 1,000 μM did not affect glucosyltransferase activity. The presence of 1,000 μM α-solanine had a small affect and 1,000 μM α-chaconine completely inhibited the reaction. Based on these data we assign the function of the gene product SGT2 as E.C. 2.4.1.173, a UDP-glucose 3-β sterol glucosyltransferase.

The effective Lenape antisense lines show a correlation with multiple T-DNA inserts and reduction in steady state levels of *Sgt2* RNA (1605, 1608 and 1619, and either reductions in α-chaconine (1605, 2-11 and 2-17) and or an increase in α-solanine (1608, 1619 and 2-18) accumulation. All of this is consistent with the conclusion that SGT2 is the primary *in vivo* solanidine glucosyltransferase in a dedicated branch of SGA biosynthesis specific for the formation of α-chaconine. Future research will focus on the combined down-regulation of *Sgt1* and *Sgt2* in concert to reduce the accumulation of both of these toxic compounds in the tubers of commercial potato cultivars.