Metabolic Study of Ginsenoside Rb_1 in Brine Shrimp (*Artemia Salina*)

Abstract

Ginsenoside Rb_1 is a 54-carbon chemical compound that is derived from ginseng, a plant that is highly valued in traditional medicine. Current research was conducted to determine if brine shrimp are capable of metabolizing ginsenoside Rb_1. Brine shrimp were grown in a brine shrimp hatchery with salt water (NaCl) at a concentration of 2.5% NaCl. Brine shrimp cultures were put in Eppendorf tubes and treated with concentrations of ginsenoside Rb_1 from 0 to 400 ppm for one day. Treatments were centrifuged, and the supernatant was filtered and used for high performance liquid chromatography (HPLC) with a C-18 column. Remaining brine shrimp were homogenized, filtered, and run through HPLC as well. Results indicate that the ginsenoside may not have been metabolized by the brine shrimp, as the supernatant had a peak for ginsenoside Rb_1 in the chromatograph and no peak for ginsenoside Rd. Further work must be conducted using a higher concentration of ginsenoside Rb_1 to confirm that brine shrimp do not digest ginsenosides.

Introduction

Ginsenosides are the active ingredients in ginseng, an herbal plant that has many medicinal uses. It is taken as a supplement and often used as a cure-all (Sivula, 2012). Ginsenoside Rd, a metabolic derivative of ginsenoside Rb_1, has been shown to slow tau protein phosphorylation (Zhang *et al*., 2014). It can also help prevent injury of SH-SY5Y (human bone marrow) cells (Liu *et al*., 2015) and heal wounds (Kim *et al*.), among various other uses.

Current study of ginsenoside Rb_1 (as pictured in Figure 1) is mostly conducted in zebrafish and other eukaryotic organisms. *E. coli* is also used for studying of the compound. *E. coli* is a prokaryote, and zebrafish are difficult to treat with ginsenosides due to the high cost of the compound.

Ginsenosides Rd and Rg_3 are metabolites of ginsenoside Rb_1 and are used to determine if the ginsenoside has progressed in the metabolic pathway. Rb_1 progresses to Rd through splicing of one glucose molecule, which progresses to Rg_3 through splicing of another glucose.

The brine shrimp is an organism that is widely favored for scientific study because they are easy to care for, do not pose an environmental hazard, require less of a chemical for treatment, do not cause many animal cruelty concerns, and are eukaryotic organisms. Thus, if brine shrimp are able to metabolize ginsenosides, they would be a preferable organism for ginsenoside study due to their small scale.

A metabolic study of brine shrimp was conducted based on a previous experiment by Wei *et al*. (2011). Wei *et al*. utilized high performance liquid chromatography (HPLC) and mass spectroscopy to detect the presence of the metabolites of the ginsenosides. A mass spectrometer could not be obtained, so only high performance liquid chromatography was conducted, using a Hewlett-Packard series 1100 HPLC machine. Data was collected using Agilent Chemstation Rev. B.04.03-SP2[108].

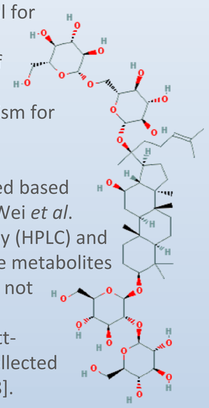

Figure 1: Ginsenoside Rb_1

Hypothesis

Brine shrimp are capable of digesting ginsenosides, and the HPLC chromatograph will have a peak for the metabolites of ginsenoside Rb_1.

Materials

- 50 mg ginsenoside Rb_1
- 1 mg ginsenoside Rd
- 5 mg ginsenoside Rg_3
- 2 L acetonitrile
- 2 L deionized water
- 15 g sodium chloride
- 5 g brine shrimp
- 2 mL formic acid
- 5 x 2 mL Eppendorf tubes
- 1 Zorbax SB-C18 column, 3.5 µm, 4.6 x 150 mm
- 20 mL 90% methanol
- 20 mL 100% dimethyl sulfoxide (DMSO)
- 40 mL Phosphate Buffered Saline (PBS)

Methods

HPLC solvents were prepared by adding 0.5 mL formic acid to water and acetonitrile in volumetric flasks and filling the volumetric flasks with the respective solvents to the 1 L mark. Solvents were filtered using a vacuum pump and 0.45 µm filter (Figure 2). 5 g brine shrimp eggs were placed in a brine shrimp hatchery with a 25,000 ppm saline salt solution. Brine shrimp were given two days to hatch. After hatching, 5 x 0.5 mL of approximately 1500 brine shrimp/mL salt water (15 brine shrimp in 10 µL) was obtained from the hatchery and placed in five 2 mL Eppendorf tubes. 0.5 mL 50, 100, 200, and 400 ppm ginsenoside Rb_1 in 1% DMSO were added to the brine shrimp and treated on a bench-top shaker. After 1 day of treatment, the samples were centrifuged at 16670 rpm. As much supernatant as possible was removed with a P-1000 pipet and transferred to an Eppendorf tube and filtered into HPLC vials to use as samples. The remaining 3 mL brine shrimp were then transferred to small falcon tubes with 7 mL PBS. The solutions of brine shrimp and PBS were then homogenized with a Tissue Tearor 985 370. The resulting solutions were filtered through a 5 µm filter into HPLC vials. The samples were then run through HPLC with 0.05% aqueous formic acid as solvent A and 0.05% acetonitrile formic acid as solvent B on a step gradient from 0% solvent B to 80% solvent B. The full gradient is shown in Table 1.

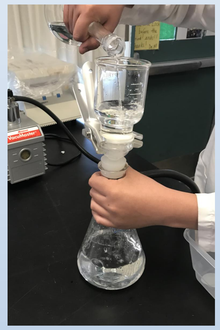

Figure 2: Filtration apparatus for high performance liquid chromatography.

Figure 3: Brine shrimp hatchery apparatus.

Results

Table 1: High performance liquid chromatography method, time by gradient

Time (min)	Gradient (% B)
0–1	0
1–11	0–7
11–13	7–17
13–17	17–20
17–33	20–21
33–41	21
41–56	21–29
56–66	29–35
67–71	35–65
71–86	65–80
86–91	80
91–96	80–0
96–101	0

Table 2: Experimental retention times for ginsenosides.

Presumed Ginsenoside	Average Retention Time
Ginsenoside Rb_1	69.98
Ginsenoside Rd	68.77
Ginsenoside Rg_3	70.73

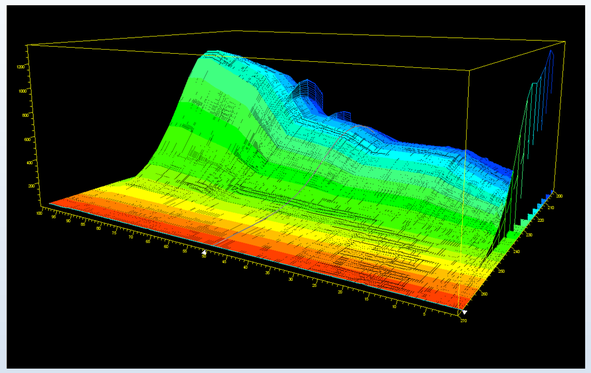

Figure 4: 3D spectrum plot of 1000 ppm ginsenoside Rd.

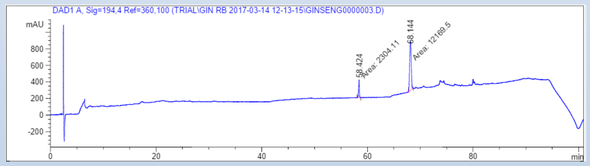

Figure 5: Experimental peak for ginsenoside Rd. First peak at 58.424 min is most likely due to sample deterioration. 68.14 presumably ginsenoside Rd.

Discussion

It was observed that brine shrimp treated with ginsenosides were qualitatively more active than the ones that were not treated. Brine shrimp treated with more ginsenoside (100, 200 ppm) were significantly more active than the brine shrimp control. Though this data is qualitative, it may indicate that further research may be necessary to verify if ginsenosides may allow brine shrimp to be more resistant to chemicals such as dimethyl sulfoxide.

As expected, retention time for ginsenoside Rb_1 was 69.98 minutes with a C-18 column. This follows closely the time presented in Wei *et al.*'s experiment, which was 48.83 minutes at a higher flow rate of 1 mL per minute (2011). Flow rate and retention time for identical methods are inversely related, so the theoretical retention time would be 69.76 minutes.

Concentration of ginsenoside Rb_1 in the brine shrimp samples was too low for proper detection in HPLC. Sample deterioration most likely posed an issue, as it is recommended to store ginsenosides in solution for no more than a day. Ginsenoside was verified as being present in some samples, but peaks for possible metabolites were too small to identify as peaks rather than noise. Data indicates that the ginsenoside may not have been metabolized, as peaks for metabolic derivatives of ginsenoside Rb_1 in the samples did not appear.

Conclusion

Brine shrimp may or may not be able to metabolize ginsenosides, but the ginsenoside concentration was not enough to definitively determine whether or not it was metabolized.

Further Work

To determine whether or not the brine shrimp may metabolize ginsenosides, the experiment should be repeated using higher concentrations of ginsenosides. Brine shrimp extraction method could also be improved by filtering out the brine shrimp rather than manually using a pipet to remove the supernatant.

References

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