Study on Antibodies against Salvinorin A and Wogonin Glucuronide for Immunoassays

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[Introduction] *Salvia divinorum* produces salvinorin A (Sal A), which is a psychoactive and potent κ-opioid agonist, also a potent drug candidate for the treatment of diarrhea, pain, headache, rheumatism and inflammatory disorders and migraine. On the other hand, wogonin glucuronide (Wgn) is one of the main compounds in *Scutellaria baicalensis*. It shows a variety of actions such as anti-hypersensitive, mitigating, anti-HIV, anti-tumor, anti-oxidant, anti-viral activities, and free radical scavenging effects.

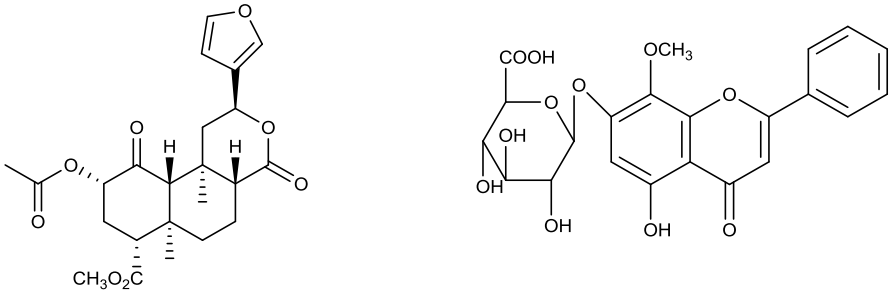

Fig. 1: Structure of salvinorin A and wogonin glucuronide

The purpose of this study is to develop convenient immunoassays for Sal A and Wgn in two kinds of Lamiaceae plants, *S. divinorum* and *S. baicalensis* using monoclonal antibodies (MAbs) and different two recombinant antibodies, single-chain variable fragment (scFv) and recombinant antigen binding fragment (Fab) and apply them to analysis of Sal A and Wgn in crude drugs and products using each medicinal plant.

[Results and Discussion] In order to prepare an immunogen, salvinorin B (Sal B) was oxidized with $NaIO_4$ and conjugated with a free amine in BSA to obtain Sal B-BSA conjugate. The hyper –immunized BALB/c mice with Sal B-BSA used to derive the cell clones yielded splenocytes that were fused with SP 2/0 myeloma cells by general fusion protocols using HVJ envelop. Eventually, hybridoma producing MAb reactive to Sal A were obtained. The MAb was checked for cross reactivities to different related salvinorin compounds. In addition, the immunochromatographic assay (ICA) was developed for a rapid analysis of Sal A.

In the case of preparation of MAb against Wgn, Wgn was conjugated with a free amine in BSA to obtain Wgn-BSA conjugate used as an immunogen. The hyper-immunized BALB/c mice injected with Wgn-BSA yielded splenocytes that were fused with SP2/0 myeloma cells by general fusion protocols using PEG. Hybridoma (315A) producing MAb reactive to Wgn were obtained. Then, the MAb was applied to an icELISA to analyze Wgn in *S. baicalensis*.

About preparation of recombinant antibodies for Wgn, the VH, VL, VH-CH1 and VL-CL fragment genes were successfully amplified by PCR using cDNA from hybridoma cell line 315A. The cloned VH and VL genes were assembled together to construct a gene encoding a full length recombinant antibody with flexible $(Gly_4Ser)_3$ linker by splice-overlap extension-PCR to prepare scFv. The scFv, VH-CH1 and VL-CL genes were cloned into pET28a vector, and expressed in *E. coli* BL21. Subsequently, scFv and each fragment of Fab expressed in the insoluble fractions was

purified and refolded to obtain active scFv and Fab. Then, each recombinant antibody to Wgn was characterized and applied for indirect competitive ELISA.

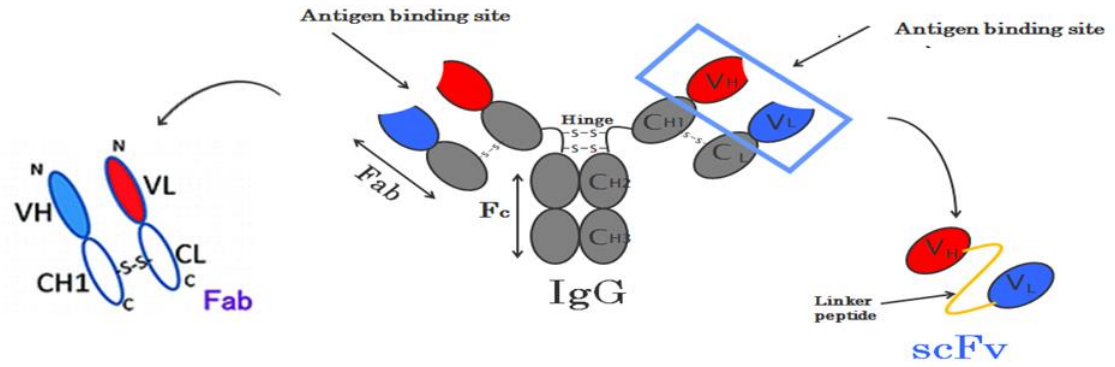

Fig. 2: Schematic drawing of a monoclonal antibody (MAb), single-chain variable fragment antibody (scFv) and antigen binding fragment (Fab)

I have successfully prepared MAbs against Sal A and Wgn and characterized the MAbs to show novel recognition to each compound. In addition, the preparation of recombinant antibodies, scFv and Fab, against Wgn were performed. Subsequently, the MAbs and each recombinant antibody have been applied to indirective competitive ELISA (icELISA) for the determination of Sal A and Wgn. The icELISA was evaluated to be a simultaneous analytical method which is useful for evaluation methods of crude drugs and a screening system of elite plants having high content of Sal A or Wgn. Moreover, ICA using MAb 3D5 against Sal A was successfully developed and applied to combined technique consists of ICA and icELISA for differentiation of *S. divinorum*. *S. divinorum* is recognized as an abused drug in many countries and the rapid immunoanalytical system for Sal A is useful to prevent the abuse of this plant.

[**Conclusion**] In conclusion, the immunoassays for Sal A and Wgn developed in this research are sensitive, reliable and convenient for qualitative and quantitative analyses for Sal A and Wgn, respectively. The methods could be applicable for various studies regarding breeding of valuable medicinal plants and efficient evaluation of crude drugs and plant products.

[**Publications**]

1. **Paudel M.K.**, Shirota O., Sasaki-Tabata K., Tanaka H., Sekita S., Morimoto S., Development of an enzyme immunoassay using a monoclonal antibody against the psychoactive diterpenoid salvinorin A, *JOURNAL OF NATURAL PRODUCTS*, **76**(9), 1654-60 (2013).
2. **Paudel M.K.**, Seiichi S., Shirota O., Sasaki-Tabata K., Tanaka H., Sekita S., Morimoto S., An immunochromatographic assay for the rapid detection of salvinorin A, (submitted).
3. **Paudel M.K.**, Seiichi S., Huy L.V., Tanaka H., Miyamoto T., Morimoto S., Development of an immunoassay using an anti-wogonin glucuronide monoclonal antibody, (submitted).
4. **Paudel M.K.**, Seiichi S., Tanaka H., Miyamoto T., Morimoto S., Study of different peptide linker length of single chain variable fragment antibody against wogonin glucuronide, (submitted).
5. **Paudel M.K.**, Seiichi S., Tanaka H., Miyamoto T., Morimoto S., An overview and comparison of two recombinant antigen-binding fragment and antigen-binding fragment from monoclonal antibody against wogonin glucuronide, (in preparation).