Communications to the Editor

Chem. Pharm. Bull. 30(5)1907—1910(1982)

RADIOIMMUNOASSAY FOR THE DETERMINATION OF GINSENG SAPONIN, GINSENOSIDE RG_1[1)]

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Radioimmunoassay method for the determination of ginsenoside Rg_1, one of the major saponins of *Panax ginseng* root, was established. The side chain of ginsenoside Rg_1 was modified and coupled to BSA. ^{125}I-Labelled compound of ginsenoside Rg_1 was prepared from a ginsenoside Rg_1-tyramine conjugate. Immunization of rabbits with the hapten-BSA conjugate gave antiserum possessing high affinity and specificity toward ginsenoside Rg_1. Ginsenoside Rg_1 can be determined by the established radioimmunoassay method over a range of 250 pg - 10 ng per tube.

KEYWORDS—— saponin; ginsenoside; *Panax ginseng*; radioimmunoassay; dammarane

Ginseng, the root of *Panax ginseng* C.A.Meyer (Araliaceae), has been used for thousands of years as one of the most important drugs in oriental medicines. Extensive studies on the chemical constituents of ginseng have revealed that saponins consisting of dammarene type triterpenes, protopanxadiol or protopanaxatriol, are the main constituents.[3)] Pharmacological and biochemical studies with ginsenosides have proved that they are responsible for a variety of biological actvities of ginseng.[4)] Recently, Hiai *et al.* reported that ginsenosides induced secretion of ACTH from the anterior pituitary to increase plasma corticoid in intact animals.[5)] This finding may explain the main biological effects of ginseng. In spite of these important findings on the biological activities of ginsenosides, little has been known concerning the fate of ginsenoside *in vivo* except for that reported by Han *et al.*[6)] This is entirely due to the lack of a highly sensitive and specific method for the determination of ginsenosides. The available method for the determination of ginsenosides such as colorimetry,[7)] TLC,[8)] GLC,[9)] DCC (droplet counter current chromatography)[10)] and HPLC[11)] are not sensitive enough to quantify ginsenosides present in biological fluids, tissues and organs. Radioimmunoassay, originally developed for the determination of biological substances such as hormones, has become the standard method to determine drug levels in biological fluids.[12)] Radioimmunoassay is highly sensitive and specific. This method was recently applied in the field of phytochemisty to determine alkaloids and glycosides in plant tissues.[13)] This communication deals with the development of highly sensitive and specific radioimmunoassay method for ginsenoside Rg_1 (1), which can be used for the determination of ginsenoside Rg_1 present in biological fluids, tissues and organs of animals as well as for the

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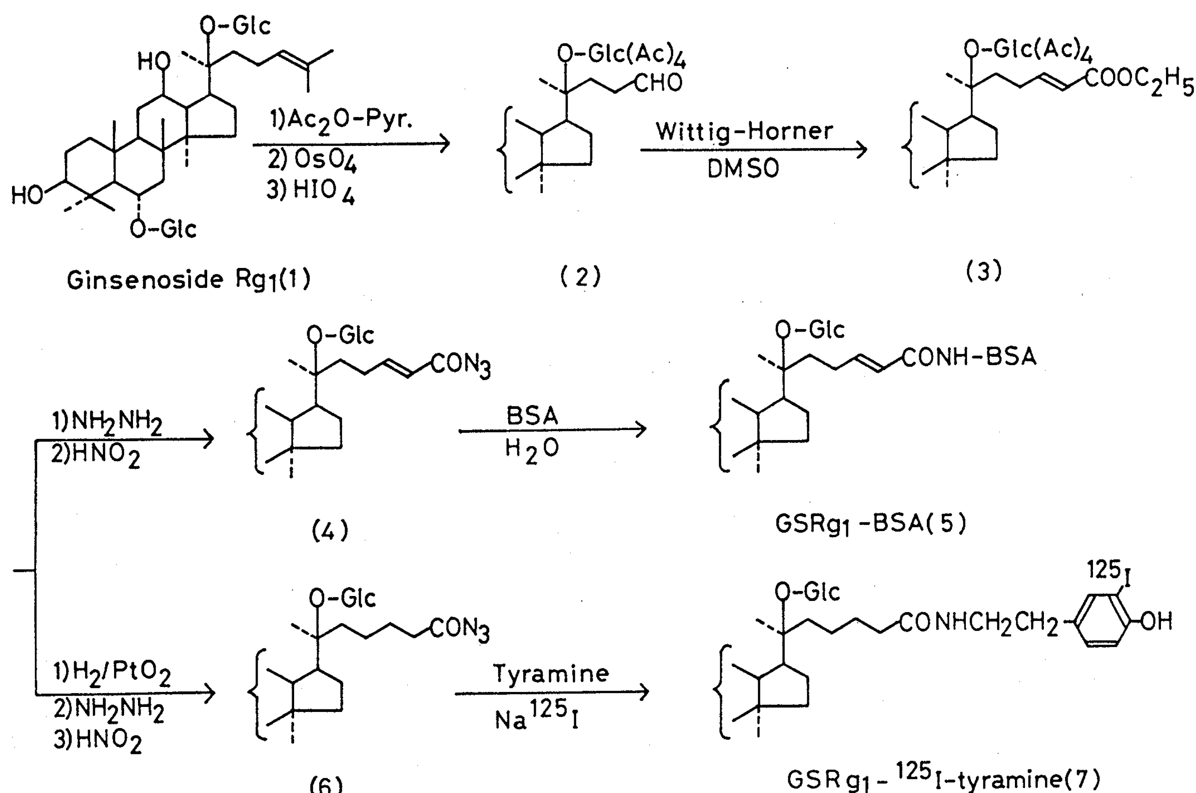

Chart 1

analysis of the saponin in plant tissues and Chinese drug preparations used for clinical treatments.

Ginsenoside Rg_1 (1) and bovine serum albumin (BSA) conjugate was prepared as shown in Chart 1. This method for the preparation of hapten-protein conjugate is applicable to all the other ginsenosides possessing dammarene type aglycones. The side chain of deca-acetyl ginsenoside Rg_1[14] was oxidized by osminium tetroxide and sodium periodate according to the procedure described by Woo[15] to give an aldehyde (2), which was further converted into an unsaturated ester (3) by the Wittig-Horner reaction. Hydrazinolysis of the unsaturated ester (3) afforded a hydrazide, which was directly converted into an azide (4) without purification to be coupled to BSA. The coupling of the azide (4) and BSA was conducted under usual conditions to give a hapten-BSA conjugate (5) that carries 3.4 - 4.6 molecules of ginsenoside Rg_1 per molecule of BSA. Immunization was performed as described previously[16] and antiserum for ginsenoside Rg_1 (1) was finally obtained after 5 months from the first immunization. In order to prepare labelled compound, the unsaturated ester (3) was hydrogenated over platinum oxide to give a saturated ester, which was treated with hydrazine to yield a saturated azide (6). This azide was treated with tyramine to give a ginsenoside Rg_1-tyramine conjugate which was labelled with carrier free $Na^{125}I$ by chloramine T method.[17] The titre of the antiserum, defined as the dilution of antiserum which binds approximately 50% of ginsenoside Rg_1-^{125}I-tyramine under the established assay condition, was 1:6400. This corresponds to a final antiserum dilution of 1:32000 in the assay tube. The radioimmunoassay (RIA) of ginsenoside Rg_1 (1) was perfomed in a polyethylene tube. A mixture of antiserum (1:6400 dilution, 0.1ml) in 0.01M phosphate buffered saline (PBS; pH 7.40) containing 0.1% lysozyme, ginsenoside Rg_1-^{125}I-tyramine (7) (15,000 cpm, 0.1 ml), PBS (0.28 ml) and a sample or standard solution (0.02 ml) was vortexed and left overnight at 4°C. Bound and free forms were separated by the double antibody method.[18] Suspension of goat antirabbit IgG immunobeads (Bio Rad) (0.2 ml) in PBS containing 0.1% BSA was added to the tube, incubated for 2 h at 37°C and centrifuged at 2000 rpm for

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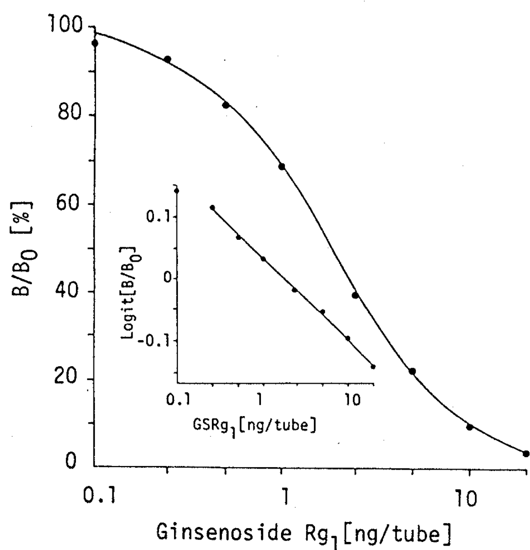


Fig. 1. Calibration Curve

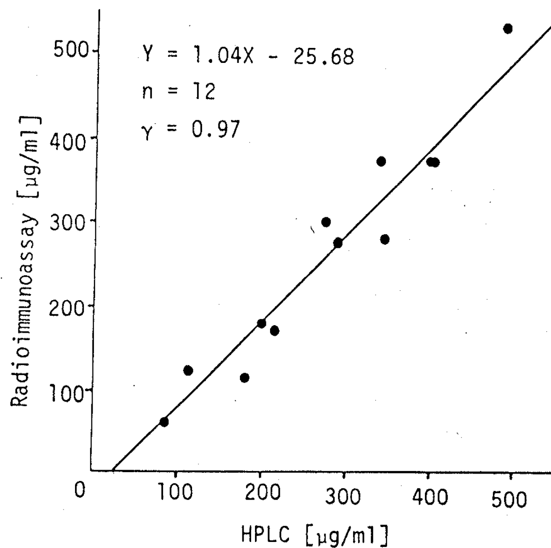
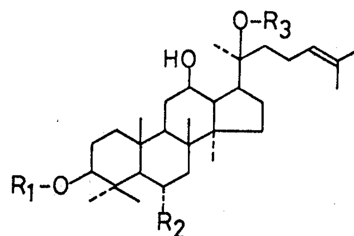


Fig. 2. Correlation between Radioimmunoassay and HPLC of Ginsenoside Rg_1

Table I. Cross Reactivity with Other Ginsenosides and Prosapogenin

Compounds	Cross reaction ratio(%)*
ginsenoside Rg_1(1)	100
Rb_1	0.58
Rc	3.41
Rd	0.98
Re	0.73
Rf	0.97
prosapogenin of $GSRg_1$	0.00

*Cross reaction ratio = ginsenoside Rg_1 concentration to induce 50% inhibition of antibody binding / sample concentration to induce 50% inhibition of antibody binding.



ginsenoside			
Rb_1	R_1= glc-glc	R_2= H	R_3= glc-glc
Rc	R_1= glc-glc	R_2= H	R_3= ara-glc
Rd	R_1= glc-glc	R_2= H	R_3= glc
Re	R_1= H	R_2= O-rha-glc	R_3= glc
Rf	R_1= H	R_2= O-glc-glc	R_3= glc
Rg_1(1)	R_1= H	R_2= O-glc	R_3= glc
prosapogenin of $GSRg_1$	R_1= H	R_2= O-glc	R_3= H

20 min. The radioactivity of the precipitated pellet that corresponded to the bound form was measured on a well type gamma-counter (Aloka JDC 751). A typical calibration curve with logit plots is shown in Figure 1, indicating that it is possible to determine the quantity of ginsenoside Rg_1 (1) in a range of 250 pg - 10 ng. This means that a 20μl sample at 12.5-500 ng/ml concentration is sufficient for the assay.

In order to evaluate the accuracy of this RIA method, the samples of varied concentrations (100-500 μg/ml) of ginsenoside Rg_1 (1) were determined by RIA and HPLC methods. HPLC was carried out with a modified silica gel column (Toyosoda LS-450 NH_2; 0.4 x 30 cm) using acetonitrile-water (80:20) as a solvent system and ginsenosides were detected with UV absorption at 203 nm. Figure 2 shows the results of determination by the two methods. A calculated correlation coefficient was 0.97. Cross reactivities with other ginsenosides and a prosapogenin of ginsenoside Rg_1 were investigated to clarify the specificity of the antiserum. As it appears in Table I all the other

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ginsenosides and a prosapogenin of ginsenoside Rg_1 showed no appreciable cross reactivity. The RIA method thus established can be used to determine the content of ginsenoside Rg_1 in plant tissues and Chinese drug preparations as well as in biological fluids of animals without any specific pretreatments of samples. These results will be reported in separate papers.

ACKNOWLEDGEMENT The authors thank Profs. J. Shoji of Showa University and O. Tanaka of Hiroshima University for their generous gifts of standard samples of ginsenosides and their derivatives. The authors (U.S. and B.H.H.) also thank the Japan Society for Promotion of Science (JSPS) for a grant for a long term visiting scientist. Thanks are also due to Dr. Yong Nam Han for his kind help in the isolation of ginsenoside Rg_1.

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(Received March 30,1982)

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