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Drug-Biomacromolecule Interaction V

Binding of Ginsenosides to Human and Bovine Serum Albumins by Fluorescence Probe Technique

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Abstract The binding properties of three ginsenosides, Rb_1, Rc and Re, to bovine and human serum albumins have been examined by fluorescence probe technique. 1-anilinonaphthalene-8-sulfonate (ANS) was used as the fluorescence probe. Protopanaxatriol glycoside, Re, did not quench the fluorescence of ANS to the bovine serum albumin. Competitive bindings between protopanaxadiol glycosides, Rb_1 and Rc, and ANS were observed. The numbers of binding sites of bovine serum albumin for Rb_1 and Rc are both 3.3. The binding constants for Rb_1 and Rc with bovine serum albumin were $1.91\times10^4M^{-1}$ and $1.04\times10^4M^{-1}$, respectively. The ginsenosides, Rb_1, Rc and Re did not quench the fluorescence of ANS bound to human serum albumin.

Keywords Ginsenosides Rb_1, Rc, Re, 1-Anilinonaphthalene-8-sulfonate(ANS), Bovine serum albumin, Human serum albumin; Fluorescence probe technique, Binding, Protein binding.

Ginseng root has been utilized for thousands of years in Korea, China and Japan as one of the most important herbal drugs in oriental medicine. Traditional oriental medicine regarded ginseng as an indispensable remedy which must be present along with other ingredients in prescriptions for, chiefly, consumption disease, all forms of debility, fatigue, stress, thirst and indigestion.[1]

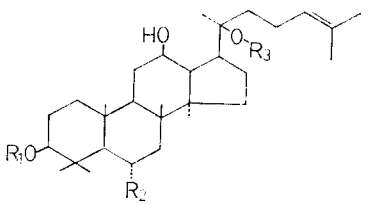
In the past 30 years scientists have paid a great deal of attention and conducted serious research in ginseng. Many scientific findings concerning the biological activities of ginseng were in good agreement with what had been claimed in oriental medicine.[2] Brekhman *et al.*[3] reviewed the divergent articles on the ginseng studies and proposed a hypothesis on the pharmacology of Panax Ginseng may exhibit an adaptogenic activity. However, the majority of published investigations in 1950's and 1960's had been performed with crude extracts from ginseng roots or pulverized ginseng roots.[2]

Many phytochemists isolated chemical ingredients from Panax Ginseng in late 1960's and early 1970's. It was learned that Panax Ginseng contains quite a number of medically useful components based on the chemical studies of ginseng.[3-7] At least nine classes of chemically distinct components such as ginseng saponins, ginseng oil and phytosterol, sugars, carbohydrates, acids, peptides, vitamins, minerals, ferments and enzymes have been isolated.[4-7] Among these, ginseng saponins are the principal constituents of the root. Accordingly, it is saponins that contribute most, if not all, of the important pharmacological activities of ginseng and ginseng extract preparations. Many investigations have

been performed with crude total saponin.[8-11]

Elyakov *et al.*[12,13] reported the isolation of six saponin glycosides(A-F). Shibata *et al.*[14-17] successfully isolated 13 ginsenosides from ginseng root extract, which were designated as ginsenosides Rx (where x=o, a, b_1, b_2, c, d, e, f, g_1, g_2, h_1, h_2). The ginseng saponins isolated by Shibata's group were quite different from the Elyakov's nomenclature. Since Elyakov *et al.* and Shibata *et al.* isolated and determined the ginseng saponin glycosides, pharmacological investigations with constituents of ginseng have been performed systematically. Takagi[18] reported that ginsenoside Rb_1, a protopanaxadiol group ginseng saponin is a central nervous system(CNS) sedative and ginsenoside Rg_1, a protopanaxatriol group ginseng saponin is a CNS stimulant. Kaku *et al.*[19] studied systematically the pharmacological properties of seven pure saponins (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1) isolated from Panax Ginseng. Chen *et al.*[20] studied pharmacokinetics of ginsenosides isolated from American ginseng in rabbit. However, the effect of binding of ginsenoside to serum albumin on the pharmacokinetics and biological activities has not been studied yet.

In this paper, the interaction of three ginsenosides, Rb_1, Rc, Re with human and bovine serum albumins is examined by fluorescence probe technique.



Rb_1: R_1=glu-2-1-glu, R_2=H, R_3=glu-6-1-glu
Rc : R_1=glu-2-1-glu, R_2=H, R_3=glu-6-1-ara
Re : R_1=H, R_2=glu-2-1-rham, R_3=Glu

EXPERIMENTAL METHODS

Materials

Ginsenosides-Rc, Rb_1 and Re isolated by Shibata's[16,17] and Han's[21] method were used. Bovine serum albumin (BSA), Fraction V and human serum albumin (HSA), Fraction V were purchased from Sigma Co. and their molecular weights were assumed to be 69,000. The concentrations of albumin solutions were determined from the absorbance of the peak at 280 nm. The molar concentration was calculated on the basis of $E^{1\%}_{1cm}=6.67$. The fluorescence probe, 1-anilinonaphthalene-8-sulfonate (ANS), was purchased from Sigma Co. All other chemicals used were of analytical reagent grade. The water used was double distilled from glass.

Apparatus

All fluorescence measurements were made with Baird-Automic Spectrophotofluorometer Model FC100 equipped with 150 watts xenon lamp and spectra were recorded on Bryans Model 2500 X-Y recoder. The entrance slit for the excitation light and the exit slit for the fluorescence emission were 32 nm, respectively. All fluorescence emission spectra in this study were uncorrected.

Fluorescence Measurement

The bindings of the probe, ANS,to HSA and BSA were determined by measuring the increase in the fluorescence intensity following the titration of the albumin solutions with the probe as described by Kim *et al.*[22-24] The HSA and BSA solutions were prepared in 0.05 M phosphate buffer at pH 7.4. Stock solution of probe was prepared using methanol at a concentration of 1×10^{-3} M. The fluorometric titrations with probe were performed at low (7.25×10^{-7}M) and high (7.25×10^{-6}M) concentration of each albumin solutions as follows: five milliliters of

each albumin solutions at high and low concentrations contained within 1 cm quartz cells were individually titrated by successive additions of 2 μl of probe solution, respectively. The titrations of the albumin solutions of low concentration were repeated in the presence of 1×10^{-4}M of Rb_1, Rc and Re, individually. The ginsenosides were added to the albumin solutions prior to titration. All fluorescence titrations in the absence and presence of ginsenosides were carried out manually with microsyringes at ambient temperature of 22±1°C. The fluorescence measurements were made at 480 nm using an excitation wavelength of 375 nm which was isosbestic for the probe-albumin binding.

Data Treatment

Enhancement of the fluorescence of the probe upon addition to albumin solution at two concentrations and the subsequent decrease of fluorescence in the presence of the ginsenosides were used to calculate the bound fraction of the probe and ginsenosides. The fraction of bound probe was calculated in a manner similar to that reported previously.[25] After the fraction of the bound probe was found for each point along the titration curve, the Scatchard equation[26] was applied to determine the binding parameters of the albumin-probe interaction as follows:

$$\frac{V}{A} = nKa - VKa$$

where V is the number of moles of bound probe per mole of protein, A is the concentration of free probe, n is the number of binding sites on the protein molecule, and Ka is the binding constant of the probe to the protein.

The competitive binding of ginsenosides was studied using ANS as the fluorescence probe. The binding constants of ginsenosides were calculated by using the equation of Klotz *et al.*[27]

RESULTS AND DISCUSSION

Typical fluorescence spectra obtained for the binding of ANS to bovine serum albumin in the presence and absence of the ginsenosides (Rb^1, Rc) are shown in Figure 1. The fluor escence intensity of ANS-BSA complex is red-

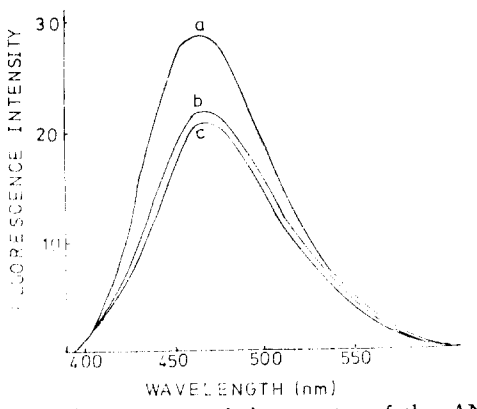


Fig. 1: Fluorescence emission spectra of the ANS-BSA complex in the presence and absence of ginsenoside.
a, in the absence of ginsenoside
b, c, in the presence of Rc, Rb_1

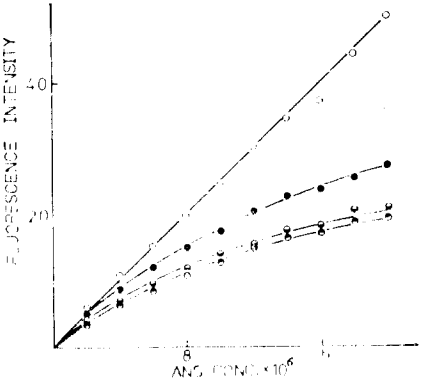


Fig. 2: Fluorescence titration curves of BSA with the ANS at high (○) and low (●) BSA concentration. Curve ◒ and ◓ are the titration curves of low BSA concentration with the ANS in the presence of Rc and Rb_1, respectively.

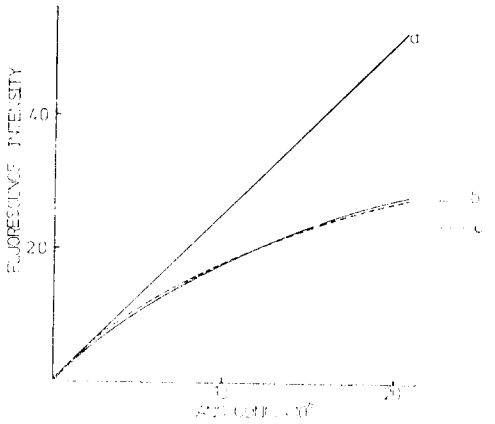


Fig. 3: Fluorescence titration curves of BSA with the ANS at high (a) and low (b) BSA concentration. Curve c is the titration curves of low BSA concentration with the ANS in the presence of Re.

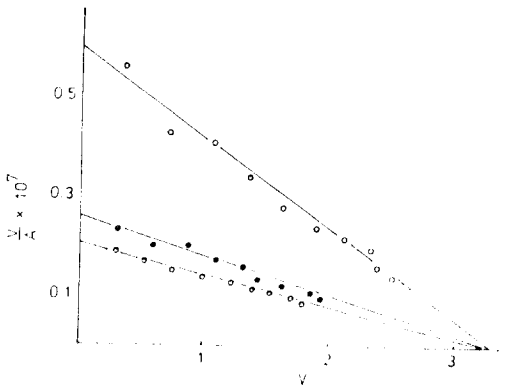


Fig. 4: Scatchard plots of the binding of the ANS to BSA.
○, in the absence of ginsenoside
●, in the presence of Re
◓, in the presence of Rb_1

uced by the addition of ginsenosides. A decrease in fluorescence of ANS-BSA complex in the presence of ginsenosides-Rb_1, Rc is the indication of the displacement of bound ANS from the BSA molecule.

Figure 2 shows the titration results of the fluorescence intensity of ANS-BSA complex in the presence of Rb_1 and Rc. Since no change of the pH and emission maxima were observed, the decrease in the fluorescence intensity of ANS-BSA complex could only be attributed to the competition between ANS and ginsenosides for the same binding sites on the BSA molecule However, Figure 3 indicates that the fluorescnce intensity of the ANS-BSA complex is not decreased in the presence of Re(curve c). This can be explained in two ways. There is possibility that Re does not bind to BSA. Also it may suggest that binding sites of Re is not identical to those of ANS. Figure 4 shows the Scatchard plots of the ANS-BSA complex in the absence and presence of fixed amount of each of the two competitive ginsenosides, Rb_1 and Rc. The concentration of BSA and ginsenosides were held constant at 7.5×10^{-7}M and 1×10^{-4}M, respectrvely, while the concentration of the probe was varied from 4×10^{-7}M. The intercepts on the abscissa are identical, but the slopes are decreased in the presence of ginsenosides. This indicates a competition between the probe and ginsenosides for the same binding sites or closely located sites on the BSA molecule. Based on the graph, the BSA molecule appeared to have 3.3 binding sites. By means of Klotz equation. the binding constants for ginsenosides Rb_1 and Rc with BSA were also estimated to be 1.91×10^{4} M^{-1} and 1.04×10^{4} M^{-1}, respectively.

The fluorescence intensity of the HSA-ANS complex was measured in the presence and absence of ginsenosides(Rb_1, Rc, Re). However, these three ginsenosides did not quenched the fluorescence of ANS bound to the HSA. This can be interpreted in two ways. First of all, ginsenosides(Rb_1, Rc, Re) may not be bound to the HSA, Secondly, it may suggest that binding sites of these ginsenosides are not identical to those of ANS.

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LITERATURE CITED

1) Huh, J.: *Compendium of Oriental Medicine.* (1610).
2) Bae, H.W. (Ed.), *Korean Ginseng*, 2nd Ed., Korea Ginseng Research Institute, Korea, 115-158 (1978).
3) Brekhman, I.I. and Dardymov, I.V.: New substances of plant origin which increase non specific resistance. *Ann. Rev. Pharmacol.* **9**, 419 (1969).
4) Shibata, S.: Active principles of ginseng roots. *Tampakushitsu* **12**, 32(1967).
5) Tanaka, O.: Chemical constituents of ginseng. *Taisha* **10**, 548 (1973).
6) Han, B.H.: in *Korean Ginseng Symposium*, Korean Society of Pharmacognosy, Seoul, Korea, 81-111 (1974).
7) Hou, J.P.: The chemical constituents of ginseng plants, Comparative Medicine East and West, Vol. V, No 22, 123-145 (1977).
8) Hong, S.A., Park, C.W., Chang, H.K., Kim, J.H., Hong, S.K. and Kim, M.S.: The effects of ginseng saponin on animal behavior. *Proceedings of International Ginseng Symposium*, 33 (1974).
9) Joo, G.N. and Lee, S.J.: Biochemical studies of ginseng saponins(IX). *Korean Biochem. J.* **10**, 59 (1977).
10) Kim, N.D. and Kim, B.K.: Effects of ginseng saponin on the intestinal absorption of sugars in rats. *J. Pharm. Soc.* (Korea) **21**, 222 (1977).
11) Kim, N.D., Kong, Y.J. and Chough, Y.S.: Influence of ginseng saponin on antipyrine induced hypothermia. *Seoul Univ. J. Pharm. Sci.* **1**, 50 (1976).
12) Elyakov, G.B. *et al.*: Glycosides from ginseng root. *Tetrahedron Lett.*, No 48, 3581 (1964).
13) Elyakov, G.B. *et al.*: Glycosides from ginseng root. *Izvest. Acad. Nauk., U.S.S.R., Otd. Ser. Khim Nauk.*, 1125, 2054 (1962).
14) Shibata, S., Tanaka, O., Soma, K., Iita, Y., Ando, T. and Nakamura, H.: Studies on saponins and sapogenins of ginseng; the structure of panaxatriol. *Tetrahedron Lett.*, No 3, 217 (1965).
15) Shibata, S. *et al.*: Protopanaxadiol, a genuine sapogenin of ginseng saponins. *Chem. Pharm. Bull.* **14**, 595 (1966).
16) Sanada, S., Kondo, N., Shoji, Jo., Tanaka, O. and Shibata, S.: Studies on the saponins of ginseng I, structures of ginsenosides Ro, Rb-1, Rb-2, Rc and Rd. *Chem. Pharm. Bull.* **22**, 421 (1974).
17) Sanada, S., Kondo, N., Shoji, J., Tanaka, O. and Shibata, S.: Studies on the saponins of ginseng II, structures of ginsenosides Re, Rf and Rg-2. *Chem. Pharm. Bull.* **22**, 2407 (1974).
18) Takagi, K.: Pharmacological studies on ginseng. *Proceedings of International Ginseng Symposium, Seoul, Korea* 119 (1974).
19) Kaku, T., Miyata, T., Uruno, T., Sako, I. and Kinoshita, A.: Chemico-pharmacological studies of saponins of *Panax ginseng* C.A. Meyer. *Arzneim. Forsch.*, 539 (1975).
20) Chen, S.E., Sawchuk, R.J. and Staba, E.J.: American ginseng III. Pharmacokinetics of ginsenosides in the rabbit. *Eur. J. Drug Met. Pharmacok.* **5**, 161 (1980).
21) Han, B.H., Han, Y.N. and Woo, L.K.: Studies on the antiinflmmatory glycosides of *Panax ginseng. J. Pharm. Soc.* (Kora) **15**, 129 (1972).
22) Kim, C.K. and Cha, H.S.: Pipethanate. HCl binding to human and bovine serum albumin using a fluorescence probe technique and circular dichroism. *Seoul Univ. J. of Pharm. Sci.* **5**, 57 (1980).
23) Kim, C.K. and Ahn, H.Y.: Drug-Biomacromle-

cule Interaction I. Physico-pharmaceutical characteristics of bovine serum albumin of ibuprofenlysine. *Arch. Pharm. Res.* **4**, 99 (1981).

24) Kim, C.K., Ahn, H.Y., Yang, J.S., Kim, Y.B. and Yu, B.S.:Drug-Biomacromolecule Interaction III: 1-anilinonaphthalene-8-sulfonate binding to bovine serum albumin by fluorescence. *Yakhak Hoeji* **26**, 85 (1982).

25) Kim, C.K., Yang, J.S. and Lim, Y.S.: Drug-biomacromolecule interaction IV. Interaction of six cephalosporins with bovine serum albumin. *Arch. Pharm. Res.* **6**, 55 (1983).

26) Scatchard, G.: *Ann. N.Y. Acad. Sci.* *51*, 660 (1949).

27) Klotz, I.M., Trwush, H. and Walker, H.M.: The binding of organic ions by proteins; competition phenomena and denaturation effects. *J. Amer. Chem. Scc.* **70**, 2935(1948).