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# Investigation of the interactions between ginsenosides and amino acids by mass spectrometry and theoretical chemistry

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ABSTRACT

In order to evaluate the essence of the interactions of ginsenosides and proteins which are composed by α-amino acids, electrospray ionization mass spectrometry was employed to study the noncovalent interactions between ginsenosides ($Rb_2$, $Rb_3$, Re, $Rg_1$ and $Rh_1$) and 18 kinds of α-amino acids (Asp, Glu, Asn, Phe, Gln, Thr, Ser, Met, Trp, Val, Gly, Ile, Ala, Leu, Pro, His, Lys and Arg). The 1:1 and 2:1 noncovalent complexes of ginsenosides and amino acids were observed in the mass spectra. The dissociation constants for the noncovalent complexes were directly calculated based on peak intensities of ginsenosides and the noncovalent complexes in the mass spectra. Based on the dissociation constants, it can be concluded that the acidic and the basic amino acids, Asp, Glu, Lys and Arg, bound to ginsenosides more strongly than other amino acids. The experimental results were verified by theoretical calculations of parameters of noncovalent interaction between ginsenoside Re and Arg which served as a representative example. Two kinds of binding forms, "head–tail" ("H–T") and "head–head" ("H–H"), were proposed to explain the interaction between ginsenosides and amino acids. And the interaction in "H–T" form was stronger than that in "H–H" form.



## 1. Introduction

Electrospray ionization mass spectrometry (ESI-MS) has been widely applied to the study of the noncovalent complexes, including the investigation of the binding selectivity, affinity and stability, identification of the binding sites and determination of the binding constants [1]. ESI-MS has already been applied to the determination of the stoichiometric relation and dissociation constants for protein–protein interactions [2–4], protein–ligand interactions [5,6], and protein–oligonucleotide interactions [7]. The stoichiometric relation of the complexes can be seen directly from the mass spectrum.

Biological functions of a protein depend directly on its noncovalent interactions with other components existing in the living system [8]. Benkestock et al. [9] investigated the interactions of HSA with warfarin, iopanoic acid and digitoxin. The three pharmaceutical molecules were chosen as site-specific probes that bound to the main sites of HSA. Schlosser et al. made one of the interacting molecules bind to magnetic beads and incubate with the target molecules in solution, then detected the free target molecules by mass spectrometry [10]. Amino acids, as the fundamental units of protein, are a class of compounds with great biochemical importance. The importance of amino acid analysis nowadays needs no emphasis, and the analysis of amino acids was carried out in many research areas, including biological and biochemical analysis, medical diagnostics, and food analysis [11]. The investigations on the interactions of amino acids and pharmaceutical molecules can reveal the nature of the interactions between proteins and pharmaceutical molecules to some extent.

For the small molecule binding determinations by ESI-MS, Kempen's group [12] investigated alkali metal cation selectivities of lariat ethers and several dibenzo-16-crown-5 lariat ethers containing methoxy, carboxylic acid, ester, or amide pendant groups with lithium, sodium, and potassium salts were studied; Blair et al. [13] evaluated the binding selectivities of caged crown ligands toward heavy metals and Schröder et al. [14] studied the dissociation behavior of Cu(urea)+ complexes by ESI-MS.

Most studies of ginsengs have been carried out with *Panax ginseng* (Asian ginseng), *Panax quinquefolius* (American ginseng) or *Panax japonicus* (Japanese ginseng). The root of *P. ginseng* has been commonly used as tonics in Eastern Asia for over 2000 years [15–18]. Ginsenosides are the main bioactive components of all kinds of ginsengs which have various clinical and pharmacological effects, such as anti-cancer activity, anti-circulatory shock effects, promotion of hematopoiesis, modulation of immune functions and cellular metabolic processes on carbohydrates, fats and proteins

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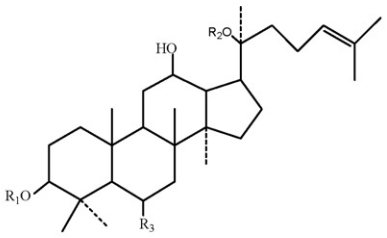


| Ginsenoside | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| $Rb_2$ | -glc(2-1)glc | -glc(6-1)ara (p) | -H |
| $Rb_3$ | -glc(2-1)glc | -glc(2-1)xyl | -H |
| Re | -H | -glc | -O-glc(2-1)glc |
| $Rg_1$ | -H | -glc | -O-glc |
| $Rh_1$ | -H | -H | -O-glc |

Glc: glucose, Ara: arabinose, Xyl: xylose.

**Fig. 1.** Structures of ginsenosides.

[19–22]. Fig. 1 shows the structures of five ginsenosides, $Rb_2$, $Rb_3$, Re, $Rg_1$ and $Rh_1$.

The noncovalent interactions of ginsenosides ($Rb_2$, $Rb_3$, Re, $Rg_1$ and $Rh_1$) and 18 kinds of α-amino acids (Asp, Glu, Asn, Phe, Gln, Thr, Ser, Met, Trp, Val, Gly, Ile, Ala, Leu, Pro, His, Lys and Arg) were investigated by electrospray ionization mass spectrometry. Because α-amino acids are the fundamental units of proteins, the interaction of the α-amino acids and pharmaceutical molecules are helpful in finding the binding sites of proteins and pharmaceutical molecules. The reasons we chose these ginsenosides were that $Rb_2$ and $Rb_3$ are isomer, and the number of the glycosyls in the ginsenosides reduces sequentially by one in the order of $Rb_2$, Re, $Rg_1$ and $Rh_1$. We tried to find the effect of ginsenoside structure on the interactions with amino acids. All the amino acid solutions were prepared with pure water without organic solvent. To keep the same experimental condition to all the amino acids, tyr and cys were not analyzed because they are difficult to dissolve in pure water. The dissociation constants of noncovalent complexes of ginsenoside and amino acid were directly calculated [23–25] based on the peak intensities of the free ginsenosides and the complexes. The noncovalent binding stoichiometric relation can be also seen directly from the mass spectrum.

With the development of modern theoretical and computational chemistry, and the improvements on the conditions of computational software and hardware, more and more molecules and interactions were investigated by theoretical computation [26–35]. In order to deeply explain the experimental results, computational chemistry was applied to the study of the noncovalent binding of ginsenoside Re and Arg, which served as a representative example.

## 2. Experimental

### 2.1. Chemicals

Amino acids used in the present investigation were purchased from Sigma Chemical Corporation. Ginsenosides, $Rb_2$, $Rb_3$, Re, $Rg_1$ and $Rh_1$, were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade methanol was obtained from Fisher Corporation, USA. Water used in all experiments was purified by Milli-Q system (Millipore Corporation, USA). All the agents were used without further purification.

Stock solutions of $1.00 \times 10^{-2}$ mol $L^{-1}$ of 18 kinds of amino acids, Asp, Glu, Asn, Phe, Gln, Thr, Ser, Met, Trp, Val, Gly, Ile, Ala, Leu, Pro, His, Lys and Arg, were prepared by dissolving corresponding amino acids in water. Ginsenoside, $Rb_2$, $Rb_3$, Re, $Rg_1$ and $Rh_1$, were dissolved in methanol ($2.76 \times 10^{-4}$ mol $L^{-1}$ $Rb_2$; $4.16 \times 10^{-4}$ mol $L^{-1}$ $Rb_3$; $2.96 \times 10^{-4}$ mol $L^{-1}$ Re; $4.31 \times 10^{-4}$ mol $L^{-1}$ $Rg_1$ and $2.97 \times 10^{-4}$ mol $L^{-1}$ $Rh_1$). The measured samples consist of 40 μL stock solution of ginsenosides, 10–80 μL amino acids of $1.00 \times 10^{-4}$ mol $L^{-1}$ and 350–280 μL water. The total volume of sample solutions was 400 μL.

### 2.2. Instrument

The mass spectra were obtained with an Applied Biosystem Q-Trap triple quadrupole mass spectrometer (Applied Biosystems Sciex, Foster City, USA) equipped with electrospray ionization (ESI) source. The instrument was joined to a computer running Applied Biosystems Analyst version 1.4 software which can record up to $m/z$ 1700.

Soft experimental conditions were important for studying noncovalent complex to avoid its dissociation. In positive mode, ionspray voltage and entrance potential were maintained at 4000 and 10 V, and declustering potential and collision energy were kept at 30 V and 10 eV. Ionspray voltage, entrance potential, declustering potential and collision energy in negative mode were maintained at −4000, −10, −30 V, 10 eV, respectively.

### 2.3. Computational methods

The ab initio Gaussian-03 program, which is a powerful and effective tool, has been widely used to characterize the electronic and structural properties of molecules, clusters and bulk solid. Here, we applied the Gaussian-03 program to perform some calculations on the interaction between ginsenoside Re and arginine. Considering the very large size of the systems and the limited computational resources, we did not calculate all the interactions, but only performed some model calculations on the representative example. Initially, the geometry of ginsenoside Re was optimized by employing STO-3G basis set by Hatree–Fock theory method (HF/STO-3G). The geometry was further optimized by using the 3–21 g basis set with Hatree–Fock method (HF/3-21G) and the 3–21 g basis set with the hybrid density functional theory method B3LYP [36–38], i.e., Becke's hybrid three-parameter exchange functional with the LYP correlation functional (B3LYP/3-21G). After geometrical optimization, detailed Natural bond Orbital (NBO) [39–41] (NPA charge distributions) analyses (NBO–B3LYP/3-21G) on the optimized structures obtained at the B3LYP/3-21G level were performed so as to get insight into the nature of experimentally observed results. All the calculations were performed with the Gaussian-03 program [42].

### 2.4. Procedures

Ginsenoside, $Rb_2$, $Rb_3$, Re, $Rg_1$ and $Rh_1$, were kept at concentrations of $2.76 \times 10^{-5}$; $4.16 \times 10^{-5}$; $2.96 \times 10^{-5}$; $4.31 \times 10^{-5}$ and $2.97 \times 10^{-5}$ mol $L^{-1}$, respectively. The constant amount ginsenosides were titrated with increasing amounts of amino acids whose concentrations were $1.00 \times 10^{-5}$, $2.00 \times 10^{-5}$, $3.00 \times 10^{-5}$, ..., $8.00 \times 10^{-5}$ mol $L^{-1}$. The mixed solutions of the ginsenosides and α-amino acids were incubated at room temperature for 30 min to reach equilibrium. After equilibrium, each sample was directly injected via a syringe pump at a rate of 5 μL $min^{-1}$ for cumulation of 2 min.