ELSEVIER

International Journal of Biological Macromolecules 37 (2005) 1–11

INTERNATIONAL JOURNAL OF
Biological
Macromolecules
STRUCTURE, FUNCTION AND INTERACTIONS

www.elsevier.com/locate/ijbiomac

# Binding of wogonin to human gammaglobulin

Yongchun Liu [a,b], Wenying He [a], Wenhua Gao [a], Zhide Hu [a,*], Xingguo Chen [a]

[a] *College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, PR China*
[b] *Department of Chemistry, Longdong University, Qingyang, Gansu 745000, PR China*

Received 31 January 2005; received in revised form 13 April 2005; accepted 19 April 2005

**Abstract**

The binding of wogonin to HGG was studied by spectroscopic method including circular dichroism (CD), fourier transformation infrared spectra (FT-IR), fluorescence spectra. The binding parameters and the thermodynamic parameters for the reaction have been calculated according to Sips method and Gibbs–Helmholtz equation, respectively, at different temperatures. AutoDock3.05 program was used to calculate the interaction modes between the drug and HGG. The Sips plots indicated that the binding of HGG to wogonin at 297, 304, 310 and 317 K is characterized by two binding sites with the average affinity constant $K_o$ at $2.102 \times 10^4$, $2.078 \times 10^4$, $1.956 \times 10^4$ and $1.931 \times 10^4$, respectively. The binding process was exothermic, spontaneous and entropy driven, as indicated by the thermodynamic analyses, and the major part of binding energy is electrostatic interaction accompanied by hydrophobic interaction and hydrogen bond. The secondary structure compositions of free HGG and its wogonin complexes were estimated by the FT-IR spectra and the curve-fitted results of amide I band, which are in good agreement with the analyses of CD spectra. Furthermore, the average binding distance between wogonin and HGG (5.60 nm) was obtained on the basis of the theory of Förster energy transfer.

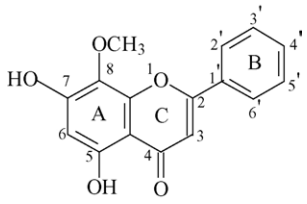

*Keywords:* Human gammaglobulin (HGG); Wogonin; Fluorescence quenching; Circular dichroism (CD); Fourier transformation infrared spectra (FT-IR); Protein; Drug

## 1. Introduction

Immunoglobulin (Ig) plays a key role in human immune response. As cell surface receptor of B lymphocyte, Ig also plays important roles in many cell actions [1]. As a familiar drug, intravenous immunoglobulin (IVIG) has been used in the treatment of primary and secondary antibody deficiencies for over 25 years [2]. Recently, great attention has been paid to IVIG potential use as adjuvant anti-neoplastic agent. Shoenfeld and Fishman [3] found that IVIG administration to mice inoculated with melanoma or sarcoma cells induced an inhibition of metastatic lung foci and prolongation of survival time. A lower number of melanoma recurrences was also demonstrated in a different model in which melanoma was induced in the footpad, followed by leg amputation. Studies in vitro have revealed that IVIGs may stimulate the production of IL-12, an anti-tumor and anti-angiogenic cytokine, and enhanced NK cell activity [3]. Shoenfeld et al. [4] reported a case of a 39-year-old man who was treated with high-dose, monthly IVIG for metastatic melanoma with radiological evidence of regression of metastases in the liver and stability of metastases in other organs. Recently studies revealed that IVIGs decreases the level of matrix metalloproteinase-9 (MMP-9) expression in the U937 monocyte line with a decrease in the m-RNA level of MMP-9 [5]. Matrix metalloproteinases are enzymes that participate in basement membrane degradation, a vital step in the invasion of metastatic cancer cells [6]. So, IgG may serve as an adjuvant therapy in cancer patients due to its anti-neoplastic properties that may synergize with more specific MMP inhibitors and other anti-cancer drugs. By using its high-safety profile, clinicians may construct new protocols. In addition, human immune gammaglobulin (HGG) is present itself in the blood of adults at 9.5–12.5 mg/mL, and as one of human plasma proteins,

* Corresponding author. Tel.: +86 931 8912540; fax: +86 931 8912582.
*E-mail addresses:* liuyongchun-001@163.com, huzd@lzu.edu.cn (Z. Hu).

0141-8130/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2005.04.005




Scheme 1. The chemical structure of wogonin.

it is capable of binding an extraordinarily diverse range of metabolites, drugs, organic compounds and relevant antigens [1]. After having been injected into blood stream, IgG accumulated itself at a high concentration with a long detention time in the organs of liver, spleen and marrow besides target tissues in human bodies [7]. With the remarkable binding properties, HGG can serve as one of important transport proteins (carrier) for drugs. HGG as a potential drug transporter may have an important role in the discovery of novel drug delivery system and targeted drug therapy. The binding of drugs to HGG also has important role in therapeutic drug monitoring as the binding may be affected by a number of drug- and patient-associated factors, resulting in altered free drug concentration and thus drug's efficacy and toxicity may be altered. Thus, the study on the binding characteristics of HGG to medical drugs is an important field in drug research and in the combination therapy.

Wogonin (Scheme 1, 5,7-dihydroxy-8-methoxy-2-phenyl-4H-benzopyran-4-one) is the main composition of Scutellariae radix (Huang-Qin in Chinese and Ogaon in Japanese), which is the root of Scrutellaria Baicalensis, has been commonly used since ancient times to treat allergic and inflammatory diseases in China and Japan, the pharmacologic actions of Scutellariae radix such as the reduction of the total cholesterol level, decrease in blood pressure, its antitoxic and antitumor actions, as well as chlolagogic, diuretic and catharic actions, have been studied. Scutellariae radix is also known to suppress the production of leukotriene C4 and D4 and to have antiallergic effects [8–10]. It is non-specific and reversible that many of these agents can bind to serum proteins, and the binding affects their pharmacological and pharmacokinetic properties [11]. Therefore a study on the binding of wogonin to HGG is very significant, but the interaction of wogonin with HGG has not been studied.

Previously, our group studied the binding of wogonin to human serum albumin [9], but albumin and γ-globulin are different proteins with different binding features to various drugs. In this paper, the binding of wogonin to HGG was firstly studied in vitro under simulated physiological conditions (pH 7.40, ionic strength 0.1) by fluorescence spectroscopy, circular dichroism (CD) and fourier transformation infrared spectra (FT-IR). The binding parameters and the thermodynamic parameters for the reaction have been calculated according to Sips method and Gibbs–Helmholtz equation, respectively, at different temperatures. The secondary structure compositions of HGG and its wogonin complexes have been estimated by the FT-IR spectra and the curve-fitted results of amide I band. AutoDock3.05 program was used to calculate the interaction modes between the drug and HGG. Furthermore, the average binding distance between wogonin and HGG was obtained on the basis of the theory of Förster energy transfer.

## 2. Materials and methods

### 2.1. Materials

Human gammaglobulin (HGG, $M_r$ 150 KDa, purity >99%) was obtained from Sigma–Aldrich Biotechnology Company. Wogonin (standard sample, purity >99.5%) was obtained from the National Institute for Control of Pharmaceutical and Products, China. 1.0 mol/L NaCl solution was used to keep the ionic strength at 0.1. Tris–HCl buffer was selected to keep the pH of the solution at 7.40. HGG solution of $1.5 \times 10^{-5}$ mol/L was prepared in pH 7.40 Tris–HCl buffer solution. Wogonin (1.0 mmol/L) solution was obtained by dissolving wogonin in ethanol. All other chemicals were of analytical reagent grade.

### 2.2. Methods

Fluorescence spectra were recorded using RF-5301PC spectrofluorophotometer (Shimadzu) with a 1-cm quartz cell. The excitation and emission bandwidths were both 5 nm. The temperature of sample was kept by recycle water throughout experiment. Absorption spectra were recorded on a Tu-1901 spectrophotometer (Beijing).

FT-IR measurements were carried out at 297 K on a Nicolet Nexus 670 FT-IR spectrometer (America) equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. All spectra were taken via the ATR method with resolution of 4 $cm^{-1}$ and 60 scans. Spectra processing procedures: spectra of buffer solution were collected at the same condition, then, subtract the absorbance of buffer solution from the spectra of sample solution to get the FT-IR spectra of proteins. The subtraction criterion was that the original spectra of protein solution between 2200 and 1800 $cm^{-1}$ was featureless [12], that is, no characteristic peaks between 2200 and 1800 $cm^{-1}$ appear and the curve is flatness. The secondary structure compositions of free HGG and its wogonin complexes were estimated by the FT-IR spectra and the curve-fitted results of amide I band.

Far-UV CD spectra were measured at 297 K on a Jasco-20c automatic recording spectropolarimeter (Japan), and the optical path length was 0.2 cm. The induced ellipticity was defined as the ellipticity of the drug-HGG mixture minus the ellipticity of drug alone at the same wavelength. CD results were expressed in terms of mean residue ellipticity (MRE) in mdeg $cm^2$ $dmol^{-1}$ according to the following equation [13]:

$$\mathrm{MRE} = \frac{\text{observed CD(mdeg)}}{C_p \times n \times l \times 10} \quad (1)$$