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Studying on inclusion complexes of Wogonin with β-cyclodextrin and hydroxypropyl-cyclodextrin

Jinxia Li[a,*], Jianbin Chao[b,**], Min Zhang[b]

[a] The Medical School of Shanxi Datong University, Datong 037009, PR China
[b] College of Chemistry and Chemical Engineering of Shanxi University, Taiyuan 030006, PR China

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ABSTRACT

The formation of the complexes of Wogonin with β-cyclodextrin (β-CD) and hydroxypropyl-cyclodextrin (HP-β-CD) was studied by fluorescence spectra and nuclear magnetic resonance spectroscopy (NMR). The formation constants (Ks) of complexes were determined by fluorescence method. The results suggested that HP-β-CD was easier to form inclusion with Wogonin than β-CD in solution. In different pH solutions, CDs have different inclusive capacity to Wo. β-CD was most suitable for inclusion of neutral form and HP-β-CD was suitable for acidic form. In addition, the experimental resulted confirmed the existence of 1:1 inclusion complex of Wogonin with CDs.

Besides, kinetic studies of DPPH• with Wogonin and CDs complexes were done. The results obtained indicated that the complex was the most reactive form. Special configuration of complex has been proposed on NMR technique.

1. Introduction

Investigations of molecular recognition have attracted much attention in supramolecular chemistry involving natural and artificial host–guest systems [1]. Cyclodextrin complex has been successfully used to improve the solubility, chemical stability and bioavailability of a number of poorly soluble compounds [2–4]. Recently, various hydrophilic, hydrophobic and ionic cyclodextrin derivatives have been utilized to extend the physicochemical properties and inclusion capacity of natural cyclodextrin [5,6]. HP-β-CD is a water-soluble derivative of β-CD, which has been widely studied as a complexion agent for many pharmaceuticals. The ability of CDs to form inclusion complexes is highly affected by size, shape, hydrophobicity and the form of the guest's molecular.

Wogonin (Wo, Fig. 1), a flavonoid present in the root of *Scutellaria baicalensis Georgi*, has attracted considerable attention because of the activities, such as anti-respiratory syncytial virus [7], anti-tumor effects [8] and anti-hepatitis B virus [9]. However, in spite of the wide spectrum of pharmacological properties, its use in pharmaceutical field is limited because of its poor solubility.

Since many guest compounds present fluorescent properties, it is interesting to analyze the changes produced in such properties when these compounds from inclusion complexes [10]. High resolution nuclear magnetic resonance (NMR) is a powerful tool for studying CD complexes [11].

The inclusion of Wogonin with biologic-molecular for example human gammaglobulin [12], human serum albumin [13] and have DNA [14] been researched by many groups, but the inclusion of Wogonin with cyclodextrins has not been investigated. Such, this paper has definite guidance purport in clinic pharmaceutics. In this paper, the interaction of Wo with β-CD and HP-β-CD were studied in detail based on Fluorescence method and NMR, and determined the effect of the complexation process on their antioxidant capacity. A new theories basis on new drug-carriers system was supplied.

2. Experimental

2.1. Apparatus and materials

Fluorescence measurements were performed by F-2500 FL spectrofluoremeter (Hitachi) using 1 cm quartz cell and both the slits were set at 20 nm with the excitation wavelength at 270 nm and the emission at 360 nm. All the NMR date was obtained on Bruker Avance DRX 300 MHZ NMR spectrometer.

The stock solution of 1.0×10^{-4} mol/L Wo (provided by Dr. Zhang and was purified by recrystallization) was prepared by dissolving and diluting its crystals in water. 2 ml of 0.2 mol/L phosphate buffer solution was used to control the pH value of the media. CDs and DPPH• were purchased from Sigma–Aldrich, Inc., St. Louis, MO. All other reagents were of analytical-reagent grade and

* Corresponding author. Tel.: +86 13935238620; fax: +86 3526033101.
** Co-Corresponding author at: The Institute of Applied Chemistry of Shanxi University, Taiyuan 030006, People's Republic of China.
E-mail address: ljx-2000@tom.com (J. Li).

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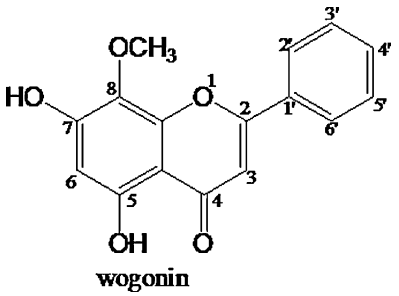

Fig. 1. The chemical structure of wogonin.

were used without purification. Doubly distilled water was used throughout. All experiments were carried out at 20 ± 1 °C.

2.2. Experimental Procedure

A 0.1 or 1 ml aliquot of the stock solution (1.0×10^{-4} mol/L) of Wo was transferred into a 10 ml volumetric burette, and an appropriate amount of 1.0×10^{-2} mol/L CDs (β-CD and HP-β-CD) was added. The solution was diluted to a final volume of 10 ml with distilled water. The mixed solution was diluted to the final volume with distilled water and ultrasonic handled for 30 min, equilibrated for 30 min at 20 ± 1 °C. The working solution was transferred into a 1 cm × 1 cm quartz cell to record fluorescence spectra. All measurements of fluorescence were made against a blank solution treated in the same way but without Wo in a 1.0 cm quartz cell.

2.3. NMR measurements

All the concentrations of Wo and CDs solution were 1.0×10^{-4} mol/L and Wo solution is diluted with CDs solutions, respectively, at the volume ratio of 1:1. ^{1}H NMR of Wo solution as well as its inclusion complexes solutions was also performed to get further evidence. D_2O was as solvent. ^{1}H NMR spectra was obtained at 300.13 MHz with 10 μs as 90° pulse width. All experiments were performed at 20 ± 1 °C.

2.4. Determination of antioxidant activity by the scavenging of the stable radical DPPH•

The antioxidant activity was measured, wherein the bleaching rate of a stable free radical, DPPH• is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH• absorbs at 517–520 nm, but upon reduction by an antioxidant or a radical species its absorption decreases.

A volume of 2 ml of 1.0×10^{-5} M DPPH• was used. Furthermore, DPPH• is insoluble in aqueous solution the scavenging study was performed in mixture of ethanol–water (20:80).

The reaction was started by addition of 1 ml of Wo (1.0×10^{-5} M), Wo/β-CD, and Wo/HP-β-CD complex samples, which correspond to the 3 mM cyclodextrin concentration. The bleaching of DPPH• was followed at 520 nm.

The decrease in absorbance at 520 nm was measured against a blank of ethanol–water (20:80) 1 ml and 2 ml 1.0×10^{-5} M DPPH• to estimate the radical scavenging capacity of each antioxidant sample. The results were expressed as percentage DPPH· elimination calculated according to the following equation [15]:

$$\mathrm{AU} = \frac{1 - A_s}{A_0} \times 100, \quad (1)$$

where AU is radical-scavenging activity, A_s is absorbance of sample and A_0 absorbance of blank sample.

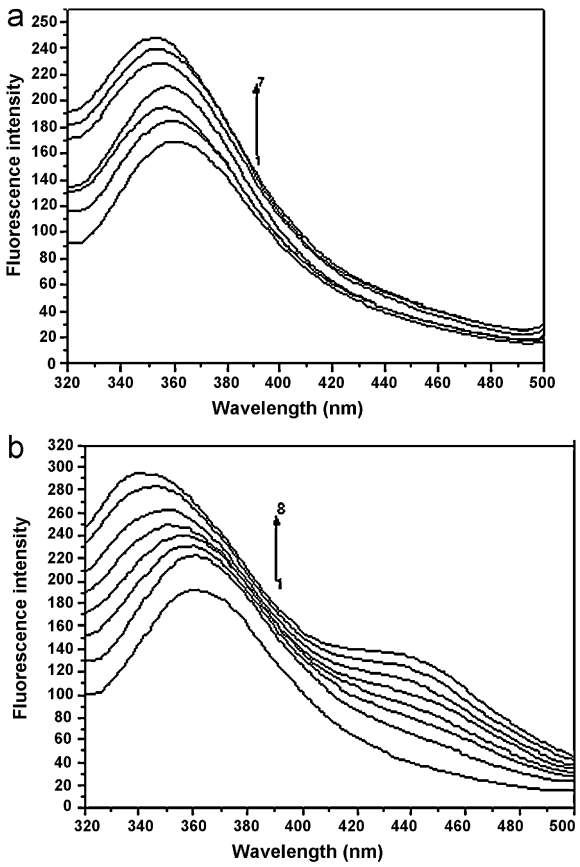

Fig. 2. Fluorescence emission spectra of 1.0×10^{-6} mol/L Wogonin in CDs. (A): β-CD; (B): HP-β-CD. CD concentration (M): β-CD: 0–6.0×10^{-3}; and HP-β-CD: 0–7.0×10^{-3}.

3. Results and discussion

3.1. Fluorescence study

Fig. 2 shows fluorescence spectra of Wogonin in the absence and presence of CDs (including β-CD, and HP-β-CD). The maximum excitation and emission wavelengths were 270 nm and 362 nm, respectively. With increasing concentration of CDs, the emission peaks appeared blue shift, and a new increasing emission wavelength was observed with the concentration increasing of HP-β-CD at about 440 nm. These suggested that the inclusion complexes were likely formed between Wo and CDs. The CDs cavities provided an apolar environment for the Wo molecular, and thus increase the fluorescence of Wo.

The inclusion formation constant (K) is a measure of the complexing power of CD. The formation constant and ratio of the complex were obtained from fluorescence data using the modified Benesi–Hildebrand equation [16]

$$\frac{1}{F - F_0} = \frac{1}{[CDs]K\alpha} + \frac{1}{\alpha} \quad (2)$$

where, F and F_0 represent the fluorescence intensity of Wogonin in the presence and absence of CDs, respectively; K is a forming constant; α is a constant.

Fig. 3 shows the double reciprocal plots of $1/(F - F_0)$ versus 1/[CD]. They exhibit good linearity. These implied that the inclusion complexes have a stoichiometry of 1:1. The binding constant (Ks) of the complexes at different pH (including pH3.50, pH6.50, pH7.50 and pH10.50) were shown in Table 1. As shown in Table 1, the binding constant and solubility of Wo determined with CDs followed the rank order HP-β-CD > β-CD. HP-β-CD was suitable for

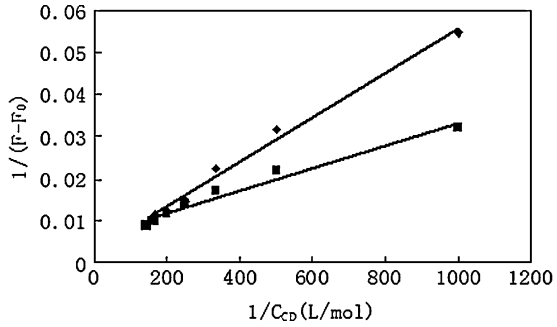

Fig. 3. The double reciprocal plots for Wogonin complexes to β-CD or HP-β-CD at pH 6.50. (♦): β-CD; and (■): HP-β-CD.

Table 1
Apparent stability constant (Ks) of Wo inclusion at different pH, the temperature is at 20 ± 1 °C.

pH	Wo/HP-β-CD		Wo/β-CD	
	K	r^2	*K*	r^2
3.50	370	0.9991	45	0.9814
6.50	220	0.9719	133	0.9990
7.50	138	0.9834	60	0.9904
10.50	72	0.9929	67	0.9855

Table 2
^{1}H NMR chemical shifts corresponding to Wo in the absence and presence of CDs in D_2O.

Wo (H)	Wo (δ0)	Wo/β-CD (δ1)	Δδ1	WO/HP-β-CD(δ2)	Δδ2
H-6	6.313	6.312	0.001	6.311	0.002
H-3	7.002	6.999	0.003	6.990	0.010
H-3′4′5′	7.629	7.628	0.001	7.626	0.003
H-2′6′	8.085	8.066	0.019	8.063	0.021

the inclusion with Wo in acidic media, and β-CD was suitable for the inclusion in neutral media. That was associated to the molecule dissociation in different pH values.

3.2. NMR

To ascertain the structure of the inclusion complexes between Wo and CDs, ^{1}H-NMR spectroscopy studies of free drug and inclusion complexes were therefore undertaken. Figs. 4 and 5 illustrated the change of hydrogen atom of Wo and CDs before and after forming the inclusion complexes. The difference in hydrogen chemical shift values between Wo in the free and complexed state were presented in Table 2. It can be seen from the figures that the H-3 and H-2′, H-6′ of Wo exhibited larger chemical shifts change after forming inclusion with β-CD, namely, the H-3 and H-2′, H-6′ of Wo were all entered into the cavity of β-CD, while H-6 and H-3′, 4′ 5′ were not into the β-CD cavity. But in the inclusion with HP-β-CD, H-6, H-3 and H-2′, H-6′ and H-3′, 4′, 5′ of Wo exhibited larger chemical shifts change, which indicated that the A–C rings of Wo were all entered into the cavity of HP-β-CD.

Table 3 showed the hydrogen chemical shift change values of CDs after forming the complexes. The H-3 of β-CD had larger

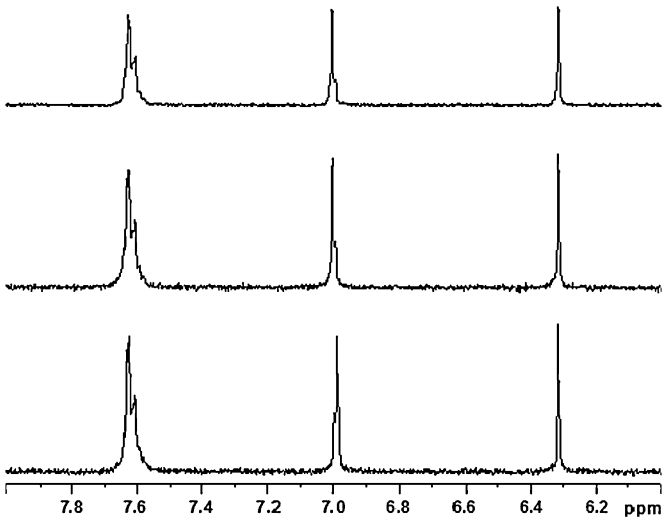

Fig. 4. ^{1}H NMR spectra of Wo and inclusion complexes: the order were Wo, Wo/β-CD and Wo/HP-β-CD from the below to the up.

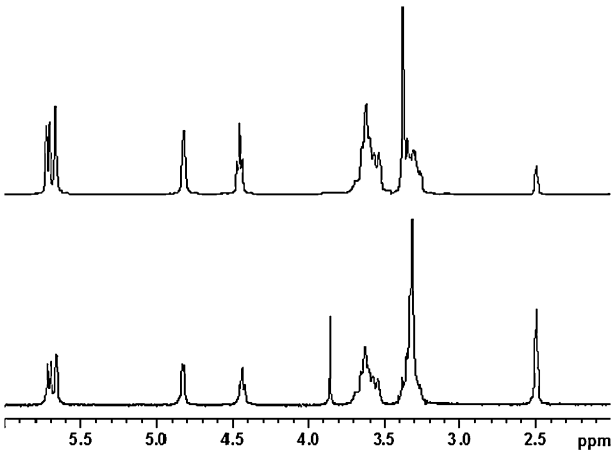

Fig. 5. ^{1}H NMR spectra of β-CD and Wo/β-CD inclusion complex from the below to the up.

chemical shift than H-5, which illustrated that the molecular of Wo entered into the cavity of β-CD from the large port; while The H-5 of HP-β-CD had larger chemical shift than H-3, which illustrated that the molecular of Wo entered into the cavity of HP-β-CD from the ventage port.

From all the above, the mechanism of complex between Wo and CDs were shown as follow (Fig. 6).

3.3. Scavenging study of DPPH• by free or complexed-Wo

DPPH• is a stable free radical generating a deep violet solution in organic solvents. Its progressive discoloration when in the presence of Wo indicated that it is acting as an antioxidant.

Table 3
^{1}H NMR chemical shifts CDs and the inclusion complexes in D_2O.

CD (H)	β-CD (δ0)	Wo/β-CD (δ1)	Δδ1	Wo-β-CD (δ0′)	Wo/HP-β-CD (δ2)	Δδ2
H-4	3.376	3.318	0.058	3.260	3.300	−0.040
H-2	3.413	3.379	0.034	3.346	3.459	−0.113
H-5	3.633	3.547	0.096	3.461	3.614	−0.153
H-6	3.696	3.632	0.064	3.615	3.762	−0.147
H-3	3.728	3.856	−0.128	3.749	3.858	−0.109

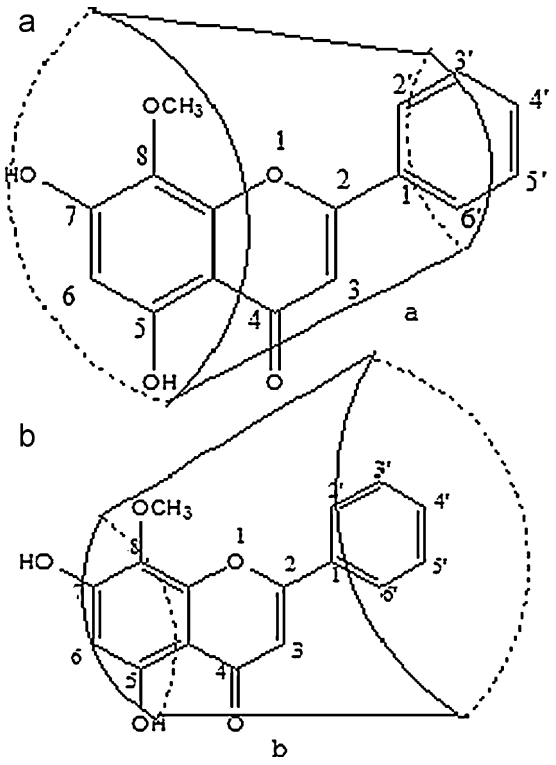

Fig. 6. The structure of inclusion complexes between Wo and CDs. (a) Wo/β-CD and (b) Wo/HP-β-CD.

Furthermore, since the mechanism of DPPH• reduction is known, the amount remaining of both reagents may be determined.

The rate of the DPPH•-scavenging reaction was measured by monitoring the decrease in absorbance at 520 nm due to DPPH•. The conclusion showed the consumption of DPPH• which indicates that the complexed Wo/CDs were more effective than free Wo, with the HP-β-CD complex (44.44%) = β-CD complex (44.44%) > free Wo (22.22%). The scavenging ability was measured as a relative scavenging in presence of free or complex Wo. That was in according with scavenging ability is related with enhanced solubility of Wo. Also theses results indicated that the complexes formed maintained the Wo antioxidant activity.

4. Conclusion

The present study has demonstrated the inclusion complex interaction between Wogonin with β-CD and HP-β-CD. The major factors of affecting guest/host binding are size matching between CD and guest, and the hydrophobicity of the guest molecule. Among the CDs examined, HP-β-CD was the most suitable for the inclusion of Wogonin in solution, β-CD was most suitable for inclusion of neutral form and HP-β-CD was suitable for acidic form. This suggested that HP-β-CD cavity supplied more hydrophobic environment. And the activity of eliminating free radical DPPH• were inclusion complexes > free Wogonin. In addition, the fluorescence spectroscopy and ^{1}H NMR data have shown the formation of a stable 1:1 stoichiometri complex of Wogonin with β-CD and HP-β-CD. Moreover, the present study demonstrated that CDs served as drugs carrier system in a dosage-controlled manner and can increase the solubility, stability and antioxidant activity of guest molecular. A mechanism was set up to expound the structure of the inclusion complexes.

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