

Structural Studies for Specific Binding Capacity of  $\beta$ -Cyclodextrin with IbuprofenWei Liu,<sup>a,c,\*</sup> Yong Zhang<sup>b</sup> and Bing Zhao<sup>c</sup><sup>a</sup>School of Pharmacy, Xinxiang Medical University, Eastern JinSui avenue, Xinxiang 453003, P. R. China<sup>b</sup>Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, P. R. China<sup>c</sup>New Drug Research and Development Center, School of Pharmaceutical Science, Zhengzhou University, No. 100, KeXue avenue, Zhengzhou 450001, P. R. China

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Ibuprofen (Ibu) and  $\beta$ -cyclodextrin ( $\beta$ CD) and its derivative (hydroxypropyl- $\beta$ -cyclodextrin, HP $\beta$ CD) complexes spatial geometry information were studied. Firstly, phase solubility experiment was carried out for S-(+)-ibuprofen (SIbu) and cyclodextrins complex. The apparent stability constant (Kc) for 1:1 complexes are 1065 M<sup>-1</sup> ( $\beta$ CD) and 1476 M<sup>-1</sup> (HP $\beta$ CD) respectively. Secondly, <sup>1</sup>H NMR and two-dimensional rotating-frame overhauser effect spectroscopy (2D ROESY) were used for binding study, and confirmed that benzene ring of Ibu is deeply included into the cavity and racemic Ibu (RSIbu) can be discriminated by  $\beta$ CD or HP $\beta$ CD. Finally, docking model was given by theoretical investigation. The model with -4.77 kcal/mol binding energy matches experimental structure.

**Keywords:** NMR; ROESY; Ibuprofen; Molecular docking; Host-guest complex.

## INTRODUCTION

Ibuprofen (Ibu) is a representative non-steroidal anti-inflammatory drug (NSAID) that was first developed as an antirheumatic drug in the 1960s.<sup>1</sup> Ibu is approved as an oral treatment for mild to moderate pain and for the reduction of fever in adults and in children.<sup>2,3</sup> Because of its safety, Ibu is used widely as a non-prescription analgesic.

Ibu is a drug molecule containing one single chiral center. It is marketed as a racemic mixture though it is known that SIbu inhibits cyclooxygenase enzymes and subsequent synthesis of prostaglandins and related compounds at peripheral sites within injured tissue.<sup>4,5</sup> Although, the R-(-)-Ibu (RIbu) can be converted to the active SIbu in vivo at the presence of an isomerase, the research still proved that half dosage SIbu is equal to racemic mixture.<sup>5</sup> More over, RIbu is harder to be cleaned out than SIbu in vivo. The research of SIbu is more significant than racemic Ibu (RSIbu).

Chiral discrimination is one of the most interesting phenomena and important domains in medicament chemistry. More than half of the drugs currently in use are chiral compounds.<sup>6</sup> Chiral cyclodextrins (CD), as the most prominent host molecules used in supramolecular chemistry, has been used widely as models for chiral molecular recognition. More over, CD can improve drugs solubility, chemical stability and bioavailability,<sup>7,8,9</sup> by forming inclusion complexes. The study of interaction between optical isomers

and  $\beta$ -cyclodextrin ( $\beta$ CD) will no doubt be important for pharmaceutical chemistry. The structure of  $\beta$ CD consists seven  $\alpha$ -D-glucose units. It is represented as a truncated cone structure with a hydrophobic cavity and external faces. The hydrophobic cavity offers the ability to form inclusion complexes by trapping foreign molecules (guest) into the cavity (host).

Here, we study the spatial geometry information of Ibu and  $\beta$ CD/HP $\beta$ CD, obtaining apparent stability constant, displaying  $\beta$ CD/HP $\beta$ CD capability of RSIbu discrimination.

## EXPERIMENTAL

## Chemical and instruments

Heavy water (D<sub>2</sub>O) was obtained from Aldrich (St. Louis). HP $\beta$ CD (degree of substitution is 6.64) and  $\beta$ CD were purchased from Deli company (Xi'an, China). SIbu and RSIbu were purchased from Furen company (Zhengzhou, China). All <sup>1</sup>H NMR spectra were recorded at 400 MHz NMR spectrometer (Bruker AVANCE III). UV electronic absorption spectra were determined using UV-2500 (Shimadzu).

## UV experiment

A series of CD solution in different concentrations from 1 to 6 mM were produced in dematerialized water. Superfluous SIbu were dissolved in dematerialized water and CD solutions. These solutions were ultrasonic dissolved

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for 30 min and placed more than 8 hours in room temperature. Liquid supernatants were diluted 50 times after centrifuging (12000 rpm) 10 min. UV spectrometer was used to detect absorbency of each solution.

### NMR experiment

The NMR spectrometer equips with a BBO probe and a variable temperature unit (VTU). The Spectra were obtained at 400.13 MHz. NMR experiment temperature was all controlled at 298 K. The solution of RSibu or Sibu with  $\beta$ CD at 1:1 stoichiometric ratio was prepared and experimentalized at former  $^1\text{H}$  NMR conditions.

Two-dimensional rotating-frame overhauser effect spectroscopy (2D ROESY) was acquired with Bruker standard parameters (pulse program roesyphsw) for the geometry of the inclusion complex. Each spectrum consisted of a matrix of 2K (F2) by 256 (F1). Size of FID covered a spectral width of 4000 Hz. The spectra were measured with a spin-lock mixing time (p15 pulse) of 200 ms, relaxation delay 2s. Gaussian apodization functions were applied in both dimensions.

### Molecular docking

The initial structures of  $\beta$ CD and Ibu were constructed using GaussView3.0. First geometry optimizations were performed to both structures using Gaussian03 in B3LYP/3-21G level. Then molecular docking was carried out in order to explain the detailed interaction mode between  $\beta$ CD and Ibu using Autodock4.2. In the docking process,  $\beta$ CD was thought as the rigid structure, and Ibu was set to be flexible in which five rotatable bonds were defined as active torsions. After grid point spacing (0.375 Å) and local search probability (0.8) setting, genetic algorithm and local search were used as conformational search method to find the reasonable donor-acceptor location. Semiempirical Autodock free energy force field, which included Van der Waals, hydrogen bond, electrostatics and desolvation, was used to estimate the interaction free energy. Docking results were analyzed, and the most possible  $\beta$ CD-Ibu complex structure is found after considering experimental data.

## RESULTS AND DISCUSSION

### Phase solubility

Phase solubility was studied in dynamic equilibrium which reached up to 8 h after ultrasonic accelerating 30 min. UV method was used to study the phase solubility of Sibu in different  $\beta$ CD or HP $\beta$ CD concentrations. The solubility value of Sibu increases with complex informing, so

that the absorptivity of Ibu increases with  $\beta$ CD or HP $\beta$ CD adding. Fig. 1 shows the relationship of Sibu solubility along with  $\beta$ CD and HP $\beta$ CD from 0 to 10 mM. The solubility of Sibu increased 7 or 9 times in  $\beta$ CD or HP $\beta$ CD solutions at 6 mM respectively. UV studies showed that the solubility of Sibu increased linearly, characteristic of a  $A_L$  type diagram.<sup>10</sup> The linear increasing indicates that Sibu and CD are combined in 1:1 stoichiometric ratio. The apparent stability constant ( $K_c$ ) for each complex was calculated based on the slope of straight-line, according to the following equation (according to Higuchi and Connors):<sup>10,11</sup>

$$K_c = \frac{\text{slope}}{S_0(1-\text{slope})}$$

$S_0$  is the intrinsic solubility of Ibu in the absence of CD. The values of apparent stability constant for a 1:1 complex are  $1065 \text{ M}^{-1}$  ( $\beta$ CD) and  $1476 \text{ M}^{-1}$  (HP $\beta$ CD) respectively.  $K_c$  values show that the process of the inclusion is easier for HP $\beta$ CD than  $\beta$ CD.

### $^1\text{H}$ NMR characterization

$\beta$ CD has a hydrophobic cavity with H-3 and H-5 inside, which are near the narrower and wider rim respectively, and H-2 and H-4 outside. H-6 is near the narrower rim and H-1 is near the wider rim. CDs offer different chemical and magnetic environment inside or outside the hydrophobic cavity, and the guest inside the cavity can also change the magnetic environment of H-3 and H-5 which are inside the cavity. So that the analysis changing of chemical shifts, including host and guest protons, can illuminate the structure of complex.<sup>12,13,14</sup>  $^1\text{H}$  NMR spectra in  $\text{D}_2\text{O}$  have been obtained in order to evaluate the effect of Ibu inclusion on the chemical shifts. Table 1 shows the displace-

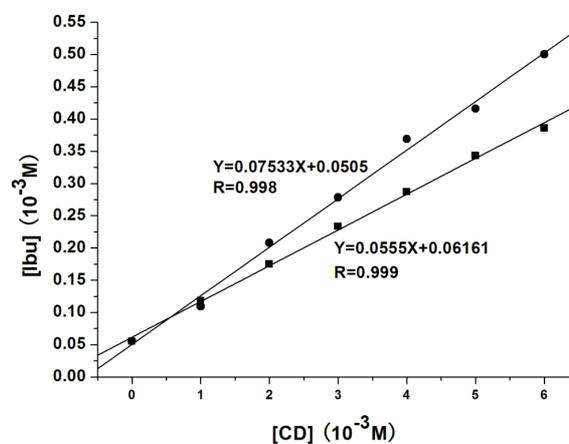


Fig. 1. Phase solubility analysis for Sibu with  $\beta$ CD (■) and HP $\beta$ CD (●).

Table 1.  $^1\text{H}$  chemical shifts of  $\beta$ -CD protons in the inclusion complexes ( $\Delta\delta = \delta_{\text{complex}} - \delta_{\text{free}}$ )

Protons of $\beta$ -CD	$\beta$ -CD free (ppm)	$\beta$ -CD complex (ppm)		$\Delta\delta$ (ppm)	
		SIbu	RSIbu	SIbu	RSIbu
H-1	4.9451	4.9298	4.9330	-0.0153	-0.0121
H-2	3.5045	3.4945	3.4990	-0.0100	-0.0055
H-3	3.8604	3.7771	3.7824	-0.0833	-0.0780
H-4	3.4410	3.4526	3.4554	0.0116	0.0144
H-5	3.7890	3.5783	3.5857	-0.2107	-0.2033
H-6	3.7850	3.7026	3.7090	-0.0824	-0.0760

ments of the chemical shifts for free  $\beta$ CD protons and  $\beta$ CD complex with SIbu or RSIbu. Upfield displacement for H-3 and 6, especially H-5, was observed clearly. H-1, 2 and 4 protons which are outside the cavity showed small chemical shifts. It suggests that aromatic ring of Ibu which is rich in  $\pi$  electrons is included into  $\beta$ CD hydrophobic cavity.

In the presence of  $\beta$ CD, chemical shifts of all SIbu protons, which serial number is showed in the insert of Fig. 3, were changed to upfield (H-e, f, h, i) or downfield (H-a, j, k) compared to the pure drug (showed in Table 2). The upfield displacement protons indicated that this moiety was located in a rich electronic density environment that produced a shielding effect. It suggests that the aromatic ring (H-e, f) may have been included into the  $\beta$ CD hydrophobic cavity. A lower upfield displacement indicates that H-h and H-i were still buried into the  $\beta$ CD cavity, but were closer to the molecule torus rim. SIbu protons chemical shifts in HP $\beta$ CD were same to in  $\beta$ CD except H-h and i which were downfield displacement. It means that H-h and i of SIbu in  $\beta$ CD are richer in  $\pi$  electrons than in HP $\beta$ CD.

Fig. 2 shows NMR signals of free Ibu and each com-

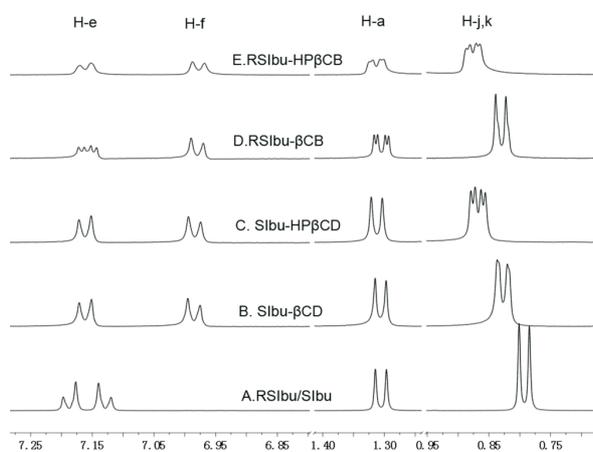
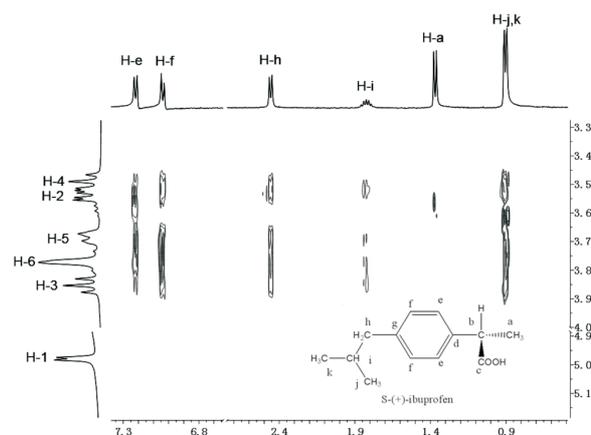

 Fig. 2.  $^1\text{H}$  NMR for free Ibu and host-guest complexes.

 Table 2.  $^1\text{H}$  chemical shifts of ibuprofen protons in the inclusion complexes ( $\Delta\delta = \delta_{\text{complex}} - \delta_{\text{free}}$ )

Protons of Ibu	SIbu free (ppm)	SIbu complex (ppm)		$\Delta\delta$ (ppm)	
		$\beta$ CD	HP $\beta$ CD	$\beta$ CD	HP $\beta$ CD
H-a	1.3054	1.3059	1.3122	0.0005	0.0068
H-b	3.5288	—	—	—	—
H-e	7.1868	7.1610	7.1615	-0.0258	-0.0253
H-f	7.1296	6.9849	6.9840	-0.1447	-0.1456
H-h	2.3959	2.3907	2.4044	-0.0052	0.0085
H-i	1.7556	1.7529	1.7870	-0.0027	0.0314
H-j	0.7926	0.8282	0.8710	0.0356	0.0784
H-k	0.7926	0.8252	0.8642	0.0326	0.0716

plex. Free racemic mixture could not be discriminated (Fig. 2A) by NMR directly. However the use of chiral recognition agent such as CD can help to resolve the matter. H-a proton of RSIbu is split in the present of  $\beta$ CD and HP $\beta$ CD, and H-e proton is split in present of  $\beta$ CD (Fig. 2D and E). This phenomenon shows that SIbu and RIbu are different in combination with  $\beta$ CD or HP $\beta$ CD. H-a proton of SIbu and RIbu are in different magnetic environment, while H-e proton of SIbu and RIbu are in the same magnetic environment. It provides theory proof for racemic mixture discrimination and separation by  $\beta$ CD or HP $\beta$ CD. We also seen H-j and k protons of SIbu been discriminated in the present of HP $\beta$ CD according to the peaks split. We thought the steric effect that lead the H-j and k protons to spin slower and caused the magnetic environment different, so that the chemical shifts of H-j and k protons are discriminated. H-j and k protons of SIbu are also in different magnetic envi-


 Fig. 3. Expansion 2D ROESY spectrum of SIbu- $\beta$ CD complex. F1 axis shows protons H-3, H-5 and H-6 of  $\beta$ CD, and F2 axis shows protons H-e, f, h, i, a, k and j of SIbu.

ronment in present of  $\beta$ CD. However discrimination capability is lower in present of  $\beta$ CD than HP $\beta$ CD because the peak of H-j and k of Sibu is only wider but not split.

### 2D ROESY experimental

More detail spatial interaction and corresponding three-dimensional geometry information between host and guest complex are given by ROESY spectra. The cross peaks for protons, which the corresponding internuclear distance is smaller than 3~4 Å, can be observed in 2D ROESY. Fig. 3 shows the expansion of the ROESY spectrum. We do not study the H-b proton of Sibu interaction with  $\beta$ CD because H-b proton is covered by  $\beta$ CD protons peaks. Cross peaks between H-e and H-f protons of Sibu and H-3, 5 and 6 protons of  $\beta$ CD were found, indicating that aromatic protons of Sibu are deeply included into  $\beta$ CD cavity. Cross peaks between H-h and i protons of Sibu and H-3, 5 and 6 protons of  $\beta$ CD were also found, however the cross peak intensity of H-h is larger than of H-i proton. This suggests H-i proton is closer to the median line of cavity, thus far away from H-3, 5 and 6, and H-h is closer to H-3, 5, and 6. Cross peaks between H-j, k and H-3, 5 and 6 suggest that methyl group is also inserted into the  $\beta$ CD cavity. Cross peak between Sibu proton and H-6 suggest that H-e to k are close to the narrower rim. None cross peak was found between H-a and H-3, 5, and 6, that suggests H-a proton is outside  $\beta$ CD cavity and near the wider rim.

### Theoretical Investigation

Configurations of Optimized 1:1 inclusion complex obtained from Autodock4.2 computation. Estimated free energy of binding is composed of four parts: final intermolecular energy (vdw, Hbond, desolv and electrostatic Energy), final total internal energy, torsional free energy and unbound system's energy. The free energy of binding we computed suggest that the most stable complex structure is the docking model that with a lower binding energy -4.77 kcal/mol. Isobutyl is near the narrower rim. This explains the NOE correction between H-6 and H-j, k. The docking model is also show that the benzene ring of Sibu is deeply included into the  $\beta$ CD cavity and other parts are outside the cavity. This explains the changes of chemical shift due to their mutual hydrophobic interaction on inclusion of Sibu into the  $\beta$ CD cavity.

### CONCLUSIONS

Phase solubility experiment shows that Sibu forms aqueous soluble 1:1 type complexes with  $\beta$ CD or HP $\beta$ CD. HP $\beta$ CD solution brings solubility increasing higher than  $\beta$ CD. The values of  $K_c$  for 1:1 complex are 1065 M<sup>-1</sup> ( $\beta$ CD) and 1476 M<sup>-1</sup> (HP $\beta$ CD), respectively. The different of  $K_c$  values suggest the different inclusion process. The <sup>1</sup>H NMR shows different inclusion structure of Sibu- $\beta$ CD and Sibu-HP $\beta$ CD complexes. <sup>1</sup>H NMR also shows H-a and H-e of RSibu are split. This means Ribu and Sibu are discriminated by HP $\beta$ CD or  $\beta$ CD. <sup>1</sup>H NMR and ROESY show aromatic nucleus of Ibu is included into  $\beta$ CD cavity, and theoretical investigation manifest that Sibu- $\beta$ CD docking model with -4.77 kcal/mol binding energy matches experimental structure.

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