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Structural Studies for Specific Binding Capacity of β-Cyclodextrin with Ibuprofen

Wei Liu,[a,c]* Yong Zhang[b] and Bing Zhao[c]

[a]*School of Pharmacy, Xinxiang Medical University, Eastern JinSui avenue, Xinxiang 453003, P. R. China*
[b]*Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, P. R. China*
[c]*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 β-cyclodextrin (βCD) and its derivative (hydroxypropyl-β-cyclodextrin, HPβCD) complexes spatial geometry information were studyed. 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-1 (βCD) and 1476 M-1 (HPβCD) respectively. Secondly, ^{1}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 βCD or HPβCD. 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.[1] Ibu is approved as an oral treatment for mild to moderate pain and for the reduction of fever in adults and in children.[2,3] 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.[4,5] 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.[5] 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.[6] 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.[7,8,9] by forming inclusion complexes. The study of interaction between optical isomers and β-cyclodextrin (βCD) will no doubt be important for pharmaceutical chemistry. The structure of βCD consists seven α-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 βCD/HPβCD, obtaining apparent stability constant, displaying βCD/HPβCD capability of RSIbu discrimination.

EXPERIMENTAL

Chemical and instruments

Heavy water (D_2O) was obtained from Aldrich (St. Louis). HPβCD (degree of substitution is 6.64) and βCD were purchased from Deli company (Xi'an, China). SIbu and RSIbu were purchased from Furen company (Zhengzhou, China). All ^{1}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

* Corresponding author. Tel: +86-0373-3831662; E-mail: liuw@iccas.ac.cn

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 βCD at 1:1 stoichiometric ratio was prepared and experimentalized at former ^1H 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 βCD 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 βCD and Ibu using Autodock4.2. In the docking process, βCD 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 βCD-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 βCD or HPβCD concentrations. The solubility value of SIbu increases with complex informing, so that the absorptivity of Ibu increases with βCD or HPβCD adding. Fig. 1 shows the relationship of SIbu solubility along with βCD and HPβCD from 0 to 10 mM. The solubility of SIbu increased 7 or 9 times in βCD or HPβCD solutions at 6 mM respectively. UV studies showed that the solubility of SIbu increased linearly, characteristic of a A_L type diagram.[10] 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):[10,11]

$$K_c = \frac{slope}{S_o\,(1-slope)}$$

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 M^{-1} (βCD) and 1476 M^{-1} (HPβCD) respectively. K_c values show that the process of the inclusion is easier for HPβCD than βCD.

^1H NMR characterization

βCD 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.[12,13,14] ^1H NMR spectra in D_2O have been obtained in order to evaluate the effect of Ibu inclusion on the chemical shifts. Table 1 shows the displace-

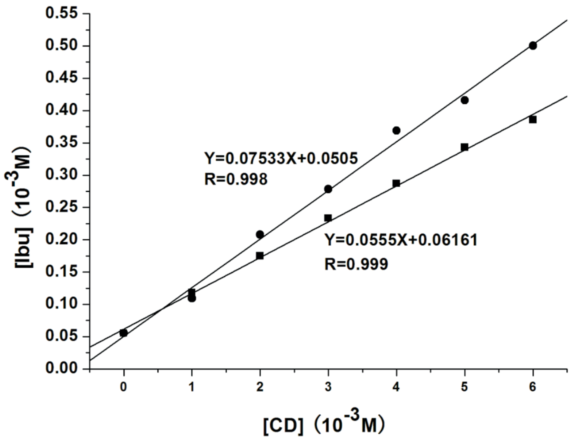

Fig. 1. Phase solubility analysis for SIbu with βCD (■) and HPβCD (●).

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

Protons of β-CD	β-CD free (ppm)	β-CD complex (ppm)		Δδ (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

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

Protons of Ibu	SIbu free (ppm)	SIbu complex (ppm)		Δδ (ppm)	
		βCD	HPβCD	βCD	HPβCD
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

ments of the chemical shifts for free βCD protons and βCD 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 π electrons is included into βCD hydrophobic cavity.

In the presence of βCD, 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 βCD hydrophobic cavity. A lower upfield displacement indicates that H-h and H-i were still buried into the βCD cavity, but were closer to the molecule torus rim. SIbu protons chemical shifts in HPβCD were same to in βCD except H-h and i which were downfield displacement. It means that H-h and i of SIbu in βCD are richer in π electrons than in HPβCD.

Fig. 2 shows NMR signals of free Ibu and each complex. 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 βCD and HPβCD, and H-e proton is split in present of βCD (Fig. 2D and E). This phenomenon shows that SIbu and RIbu are different in combination with βCD or HPβCD. 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 environmen. It provides theory proof for racemic mixture discrimination and seperation by βCD or HPβCD. We also seen H-j and k protons of SIbu been discriminated in the present of HPβCD 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-

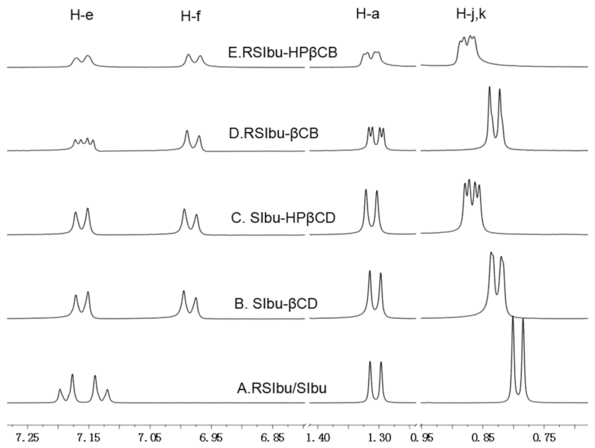

Fig. 2. [1]H NMR for free Ibu and host-guest complexes.

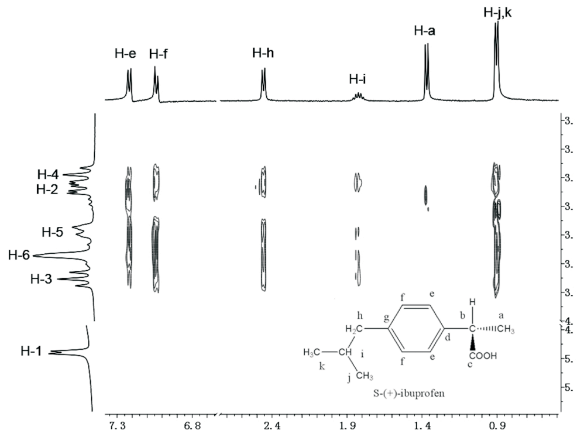

Fig. 3. Expansion 2D ROESY spectrum of SIbu-βCD complex. F1 axis shows protons H-3, H-5 and H-6 of βCD, and F2 axis shows protons H-e, f, h, i, a, k and j of SIbu.

ronment in present of βCD. However discrimination capability is lower in present of βCD than HPβCD 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 βCD because H-b proton is covered by βCD protons peaks. Cross peaks between H-e and H-f protons of SIbu and H-3, 5 and 6 protons of βCD were found, indicating that aromatic protons of SIbu are deeply included into βCD cavity. Cross peaks between H-h and i protons of SIbu and H-3, 5 and 6 protons of βCD 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 βCD 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 βCD 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 βCD 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 βCD cavity.

CONCLUSIONS

Phase solubility experiment shows that SIbu forms aqueous soluble 1:1 type complexes with βCD or HPβCD. HPβCD solution brings solubility increasing higher than βCD. The values of K_c for 1:1 complex are 1065 M^{-1} (βCD) and 1476 M^{-1} (HPβCD), respectively. The different of K_c values suggest the different inclusion process. The ^1H NMR shows different inclusion structure of SIbu-βCD and SIbu-HPβCD complexes. ^1H NMR also shows H-a and H-e of RSIbu are split. This means RIbu and SIbu are discriminated by HPβCD or βCD. ^1H NMR and ROESY show aromatic nucleus of Ibu is included into βCD cavity, and theoretical investigation manifest that SIbu-βCD docking model with -4.77 kcal/mol binding energy matches experimental structure.

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