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ORIGINAL ARTICLE

# Study of the complexation of resveratrol with cyclodextrins by spectroscopy and molecular modeling

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**Abstract** In present work the complexation of Res with two kinds of cyclodextrins (CDs), native $\beta$-cyclodextrin ($\beta$-CD) and modified hydroxypropyl-$\beta$-cyclodextrin (HP-CD), have been investigated by fluorescence spectroscopy, $^1$H-NMR spectroscopy and molecular modeling methods. The stoichiometric ratios, inclusion constants and thermodynamic parameters have been determined by the fluorescence data. In all cases 1:1 inclusion complexes are formed. The inclusion ability of HP-CD is larger than that of $\beta$-CD. Both inclusion processes have negative $\Delta G$, negative $\Delta H$ and positive $\Delta S$. Thermodynamic analysis suggests that Van der Waals force of guest-host interactions and the release of high-enthalpy water molecules from the cavity of CDs play important roles in driving complex formation. The study of molecular modeling shows that part of the A-ring and the B-ring of Res are placed in the cavity of $\beta$-CD, and the hydroxyl groups are projected outside. As for Res in HP-CD, the B-ring of Res is included in the cavity of HP-CD, and part of the A-ring is pointed outside. $^1$H-NMR spectroscopy results show that $H_2$, $H_3$, $H_4$ and $H_5$ protons of Res are more affected by the complexatin, indicating that they are located inside the torus of CDs, which are in agreement with the result of the molecular modeling.

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**Keywords** Resveratrol · Cyclodextrin · Fluorescence spectroscopy · Thermodynamic parameters · Molecular modeling · NMR spectroscopy

## Introduction

Resveratrol (trans-3, 4′, 5-trihydroxystilbene, Res) belongs to a group of naturally nonflavonoid polyphenol compounds found in plants, foods and beverages [1, 2]. Its synthesis is triggered by plant stress conditions such as injury or fungal attack [1]. In recent years, research into Res has shown that Res has many beneficial biological effects, such as modulates lipid metabolism, protects low-density lipoproteins against oxidative and free radical damage [3, 4], inhibits platelet activation and aggregation [5]. The most interesting biological activity of this compound is its strong anticarcinogenesis effect [6]. It can block the carcinogenesis stages of initiation, promotion, and progression and has potent chemopreventive effect on multiple carcinogenesis models such as skin cancer, breast cancer, lung cancer, prostate cancer, etc. [7]. Although the biological positive effects of Res are largely admitted, the poor solubility and its sensitivity to air, light and oxidant may constitute a serious problem for its bioavailability. The problem could be overcome by the formation of inclusion complexes with cyclodextrins (CDs).

In recent years, pharmaceutical applications of CDs as additive and drug-complexing agents have been growing rapidly. CDs are cyclic oligosaccharides composed of glucopyranose units and can be represented as a truncated cone structure with a hydrophobic cavity [8]. The hydrophobic cavity forms inclusion complexes with a wide range of guest molecular [9–12]. Inclusion of a drug molecular will modify the physico-chemical properties such as solubility, stability and bioavailability of slightly soluble drugs [9, 13].

Unmodified or unsubstituted $\beta$-cyclodextrin ($\beta$-CD) has poor water solubility and is parenterally unsafe due to its nephrotoxicity. Therefore, several modified and relatively safe cyclodextrins have been made, such as hydroxypropyl-$\beta$-CD (HP-CD) and sulfobutyl ether-$\beta$-cyclodextrin [7]. At present inclusion of Res with CDs has been reported in very few publications. The antioxidant activity of the Res/CD inclusion complexes has been reported by our group [14]. The apparent formation constants of the Res complexes with CDs have been studied by different methods, such as nuclear magnetic resonance (NMR), enzymatic, solubility and fluorimetric assays [15–17]. Although the solution experiments of inclusion of Res with CDs have been recognized, the exact position of Res in CDs has not been known and this can help us to get a better understanding of the formation of complexes. The present work has involved the investigation of the interaction of Res with $\beta$-CD or HP-CD by fluorescence and NMR techniques. The thermodynamic parameters have been obtained and driving forces of the including reactions have been analyzed. The exact location of Res in $\beta$-CD or HP-CD has been determined by molecular modeling method.

## Experimental

### Materials

Res was purchased from Sigma (St. Louis, MO, USA), its purity was 99% according to the manufacture. Stock solution (10 mM) of Res was prepared in 50% DMSO and kept in the dark at 277 K, the molar concentration was based on its molecular weight of 228 Da. $\beta$-CD and HP-CD (1.0 molar substitution dextrin) were purchased from Sigma Aldrich. Stock aqueous solutions of CDs (10 mM for $\beta$-CD and 20 mM for HP-CD) were prepared daily and the molar concentrations were based on their molecular weights of 1134 Da and 1540 Da, respectively. All other reagents were of analytical reagent grade. Deionized water from a Milli Q system apparatus (Millipore, USA) was used throughout the experiments.

### Preparation of the inclusion complexes

The inclusion complexes between Res and CDs were prepared by wet technologies [15]. Each CDs (0.1 mmol) was finely suspended in water (5 mL) at room temperature with vigorous stirring and an equimolar amount of Res (0.1 mmol) was directly added to the suspension. To minimize photochemical degradation the flasks were covered with aluminium foil. After stirring for 120 h, the suspensions were filtered through 0.45 μm cellulose acetate membrane filters to remove undissolved solid. The water was removed by evaporation in vacuum.

### Fluorescence measurements

Fluorescence spectroscopy measurements were performed on F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with a thermostatically controlled cell holder and a 1.0 cm quartz cell. Excitation and emission slit widths were 5 nm, the scan speed was 240 nm $min^{-1}$. The excitation wavelength of 300 nm was used, and the emission spectra were recorded from 310 to 520 nm at three temperatures (293 K, 303 K and 313 K). Res concentration was fixed at 15 μM while the CDs concentration was varied from 0 to 0.75 mM. All solutions were shaken for 4 h in a thermostated bath at three temperatures. To minimize photochemical degradation the flasks were covered with aluminium foil.

### Proton magnetic resonance ($^1$H-NMR)

Proton NMR spectra of the inclusion complexes, CDs and Res were taken on a Varian Gemini 300 MHz spectrophotometer. Chemical shifts were expressed as ppm ($\delta$). Res was dissolved in DMSO-$d_6$, the others were dissolved in $D_2O$.

### Molecular modeling

Guest–host interactions were computationally simulated in vacuo for Res/$\beta$-CD and Res/HP-CD complexations using the Tripos Force Field of the Sybyl software (release 7.0, Tripos Associates, St. Louis, Missouri, USA). The initial molecular geometry of $\beta$-CD and Res were obtained from Protein Data Bank (PDB ID: 1GVI and 1UOW, respectively), and the structure of HP-CD was constructed manually. Energy minimizations were carried out by the simplex algorithm and the conjugate gradient was used as a termination method with gradient of 0.2 kcal/mol Å. Non-bonded cut-off distance was set at 8 Å.

During the docking, both structures of Res and $\beta$-CD (HP-CD) molecules were allowed to approach each other along the symmetric $x$-axis passing through the center of the $\beta$-CD cavity by the A-ring and the B-ring sides, respectively (Fig. 1). Multiple starting positions were generated by movement of Res along the $x$-axis from $x = -5$ Å to $x = +10$ Å. The energy of each complex was computed at 2.5 Å intervals, and the structure with the lowest bind energy was chosen [18].

## Results and discussion

### Fluorescence spectroscopy studies

Figure 2 shows the fluorescence spectra of Res in the presence of $\beta$-CD or HP-CD at different concentrations.

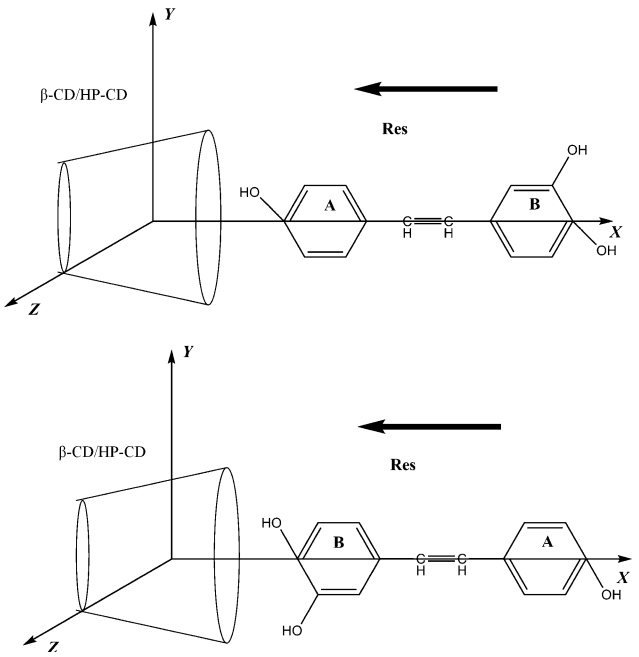



**Fig. 1** An example for the docking of Res with $\beta$-CD or HP-CD

Res has an emission peak at 394 nm with the excitation wavelength at 300 nm. With the addition of CDs, the fluorescence intensity of Res is markedly enhanced. The value of fluorescence intensity of Res with $\beta$-CD at the concentration of 0.75 mM is 2.1 times than that with water, as for Res with HP-CD at the concentration of 0.75 mM, the value is 5.3 times than that with water. Furthermore, the addition of CDs results in the distinct blue shifts of the maximum peak position. The blue shift of 6 nm (from 394 to 388 nm) by the addition of $\beta$-CD and 11 nm (from 394 to 383 nm) by the addition of HP-CD are observed. The enhancement of fluorescence intensity and the blue shifted fluorescence maximum suggest the formation of inclusion complexes between Res and $\beta$-CD or HP-CD. Analogous results were obtained with 2-amino-7-bromofluorene [19] and aflatoxin B1 [20] as the guest molecules. When the guest molecules are entrapped in the CDs cavity, the microenvironment with smaller polarity and stronger rigidity would astrict the freedom of guest molecules and increase the fluorescence quantum yield. Furthermore, the steric hindrance of CDs torus can protect the excited states from nonradiative and quenching processes that normally readily occur in bulk aqueous solution and enhance the fluorescence efficiencies of guest molecules [21].

## Stoichiometry of the inclusion complexes and inclusion constants

The fluorescence data of Res in the presence or absence of CDs can be analyzed by the Benesi–Hildebrand equation [22]:

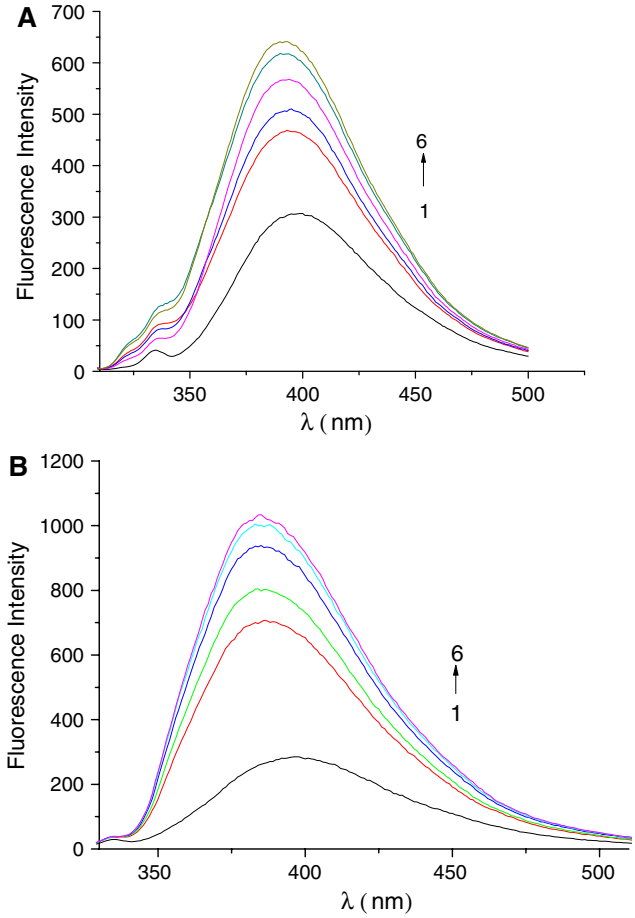



**Fig. 2** Fluorescence spectra of Res in the presence of $\beta$-CD (**A**) or HP-CD (**B**) at 298 K. Concentration of CDs(1 → 6, mM): 0, 0.15, 0.30, 0.45, 0.60, 0.75. Concentration of Res: 10 μM

$$\frac{1}{F - F_0} = \frac{1}{K(F_\infty - F_0)[CD]_0} + \frac{1}{F_\infty - F_0} \quad (1)$$

where $K$ is the inclusion constant, $F_0$ is the fluorescence intensity of Res without CDs, $F$ is that with a certain concentration of CDs, $F_\infty$ is the fluorescence intensity of Res with the highest concentration of CDs. The plot of $1/(F-F_0)$ vs. $1/[CD]_0$ is given in Fig. 3. The linearity in the plot reveals the formation of 1:1 complex between Res and $\beta$-CD or HP-CD. From the slop and intercept, the inclusion constant $K$ was calculated and listed in Table 1. As shown in Table 1, the inclusion constants $K$ of Res with $\beta$-CD or HP-CD decreases with increasing temperature, which indicates that the stability of complexes become weakened with increasing temperature, and the complexation process is favorable at low temperature. It is also shown that the inclusion constant of Res with HP-CD is larger than that of Res with $\beta$-CD, which suggests that HP-CD exhibits stronger inclusive ability than native $\beta$-CD, which implying that the cavity of modified $\beta$-CD provides a better

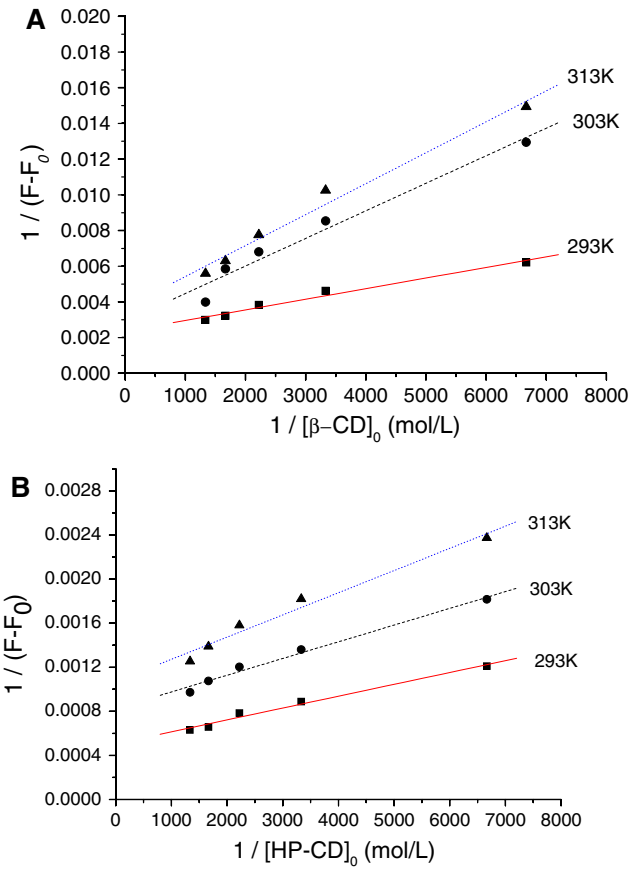



**Fig. 3** Benesi–Hildebrand plots for the complexation of Res with $\beta$-CD (**A**) or HP-CD (**B**)

**Table 1** Inclusion constants and thermodynamic parameters of Res/$\beta$-CD or Res/HP-CD inclusion complex

| $T$ (K) | $R$ | $K$ ($M^{-1}$) | $\Delta G$ (kJ $mol^{-1}$) | $\Delta H$ (kJ $mol^{-1}$) | $\Delta S$ (J $mol^{-1}$ $K^{-1}$) |
|---|---|---|---|---|---|
| *Res/β-CD* | | | | | |
| 293 | 0.9882 | 2122 | −18.66 | | |
| 303 | 0.9837 | 2038 | −19.14 | −4.52 | 48.25 |
| 313 | 0.9882 | 1884 | −19.62 | | |
| *Res/HP-CD* | | | | | |
| 293 | 0.9922 | 6015 | −21.21 | | |
| 303 | 0.9931 | 5438 | −21.62 | −9.27 | 40.76 |
| 313 | 0.9877 | 4714 | −22.03 | | |

protective microenvironment. Strong inclusive ability probably can be understood that the substitution by hydroxypropyl leads to the enlargement of the bigger opening of native $\beta$-CD and the destroy the strong intramolecular hydrogen bond network, which lets guest molecular access the HP-CD cavity easily and give a higher stability constant [8].

## Thermodynamic parameters and driving forces of the including reactions

The thermodynamic parameters of the inclusion processes can be obtained from the temperature dependence of the inclusion constants of the complexes. If the enthalpy change ($\Delta H$) does not vary significantly over the temperature range studied, then the thermodynamic parameters of enthalpy change ($\Delta H$), entropy change ($\Delta S$) and free energy ($\Delta G$) can be determined from the Van't Hoff equation:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (2)$$

where $K$ is the inclusion constant at the corresponding temperature and $R$ is the gas constant. The $\Delta H$ and $\Delta S$ are determined from the linear Van't Hoff plots, as shown in Fig. 4. The $\Delta G$ is estimated from the following equation:

$$\Delta G = \Delta H - T\,\Delta S \quad (3)$$

The results of $\Delta H$, $\Delta S$ and $\Delta G$ are also presented in Table 1. Both inclusion processes have negative $\Delta G$ and negative $\Delta H$, which indicates that the inclusion process of Res with $\beta$-CD or HP-CD is spontaneous and the formation of complex is exothermic reaction. The inclusion process of Res with HP-CD is slightly more thermodynamicly favored than that of Res with $\beta$-CD. The negative $\Delta H$ suggests that Van der Waals force of guest–host interactions and the release of high-enthalpy water molecules from the cavity of CDs play important roles in driving complex formation [8, 12]. $\Delta S > 0$ correspond to guest that penetrate into the cavity. The entropy increases is mainly due to the disorder caused by the breakage of the ordered solvent shell around the guest or inside the CD cavity [12].

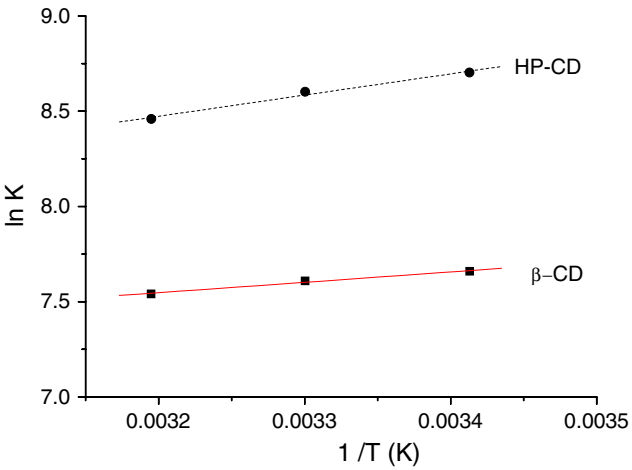



**Fig. 4** Van't Hoff plots for the formation of complexes

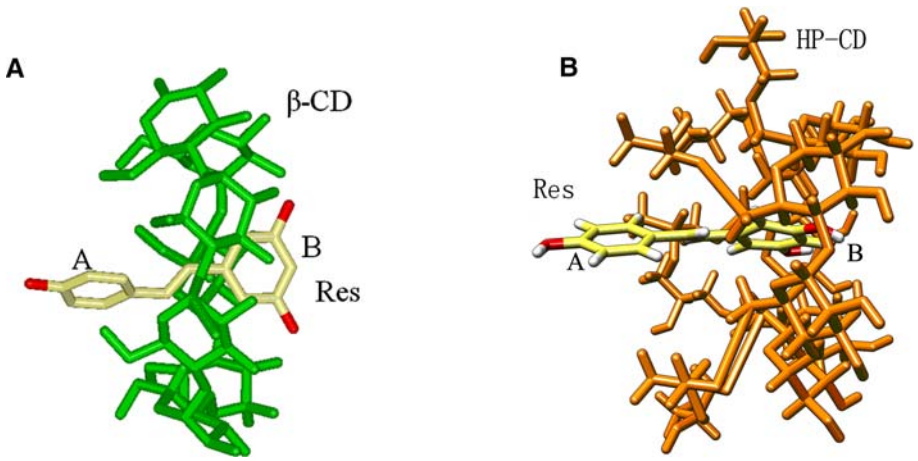



**Fig. 5** Optimized structure with the lowest binding energy of Res/$\beta$-CD (**A**) or Res/HP-CD (**B**) inclusion complex

## Molecular modeling

In order to gain further insights into the inclusion processes, it is important to characterize the orientation of Res molecule inside the CDs cavity. The optimized geometry with the lowest bind energy is depicted in Fig. 5. According to the calculation, there is no possibility of accommodation of Res molecule completely in the cavity of $\beta$-CD with a length of 7.8 Å. So the complex formed is the axial inclusion complex, part of the A-ring and the B-ring of Res are placed in the cavity of $\beta$-CD, and the hydroxyl groups are projected outside. The simulated result is coincident with the previous reports that $\beta$-CD with a hydrophobic cavity of inner diameter 6.2 Å can include the less polar moiety of the guest molecules [18, 20]. According to the simulation, the B-ring of Res is included in the cavity of HP-CD, and part of the A-ring pointed outside, whose hydroxyl group interact with the hydrophilic environment.

## $^1$H-NMR spectroscopy studies

The NMR-techniques are commonly used to study inclusion complexes of CDs. The $^1$H-NMR spectra of Res and CDs free and complexed were first reported by Bertacche [15]. In Fig. 6 is shown the $^1$H-NMR spectra of Res free and complexed by our experiment. The chemical shifts of Res are summarized in Table 2. The shifts of $H_2$, $H_3$, $H_4$ and $H_5$ were by far the most affected. From the results of molecular modeling $H_2$, $H_3$, $H_4$ and $H_5$ protons of complexed Res are located inside the torus of CDs. $H_1$ and $H_6$ were shifted less by the inclusion process, and they are located on the cavity rim of CDs from the results of molecular modeling.

## Conclusion

The limited water solubility of Res can be overcome by the formation of inclusion complexes with CDs. The complexes formations between Res and $\beta$-CD or HP-CD are studied by fluorescence spectroscopy, $^1$H-NMR spectroscopy and molecular modeling. Both of complexes are 1:1 stoichiometry. The inclusion constant of Res with HP-CD is larger than that of Res with $\beta$-CD, which suggests that HP-CD exhibits stronger inclusive ability than native $\beta$-CD. Thermodynamic analysis suggests that Van der Waals force of guest–host interactions and the release of high-enthalpy water molecules

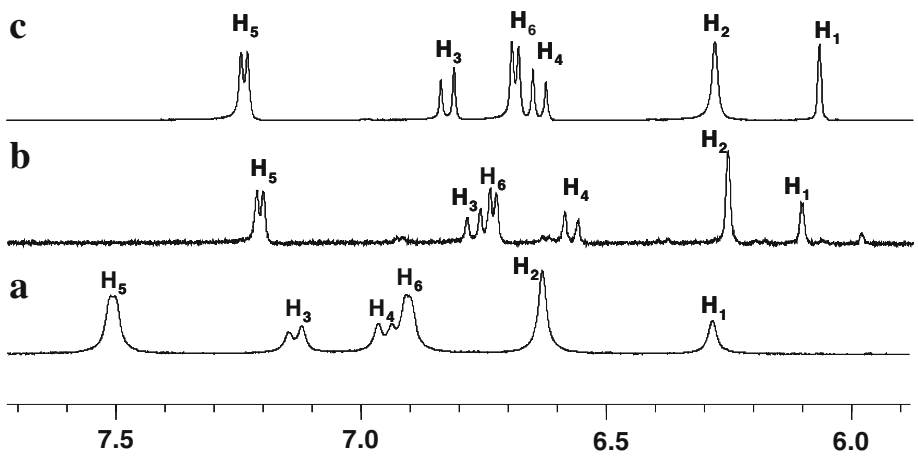
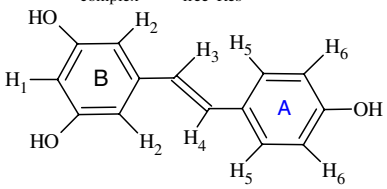



**Fig. 6** $^1$H-NMR spectra of free Res (**a**), Res/$\beta$-CD inclusion complex (**b**) and Res/HP-CD inclusion complex (**c**)

**Table 2** $^1$H-NMR chemical shift displacement ($\triangle\delta$, ppm) of Res by complexation with $\beta$-CD or HP-CD. $\triangle\delta$ were expressed as $\triangle\delta = \delta_{complex} - \delta_{free\ Res}$




| Proton | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ |
|---|---|---|---|---|---|---|
| With $\beta$-CD | −0.154 | −0.348 | −0.332 | −0.348 | −0.264 | +0.127 |
| With HP-CD | −0.189 | −0.321 | −0.277 | −0.284 | −0.238 | +0.184 |

from the cavity of CDs play important roles in driving complex formation. Molecular modeling result shows that the complex formed is the axial inclusion complex, part of the A-ring and the B-ring of Res are placed in the cavity of $\beta$-CD, and the hydroxyl groups are projected outside. For Res with HP-CD, the B-ring of Res is included in the cavity of HP-CD, and part of the A-ring is pointed outside. $^1$H-NMR spectroscopy results show that $H_2$, $H_3$, $H_4$ and $H_5$ protons of Res are more affected by the complexation, indicating they are located inside the torus of CDs. This fact is in good agreement with the result of molecular modeling.

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