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Structural Studies on Host + Guest Recognition Sensory Systems

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Abstract. Rhodamine B-ethylenediamine-β-cyclodextrins (RhB-β-CDen) and rhodamine B-β-cyclodextrins (RhB-β-CD) can form inclusive complexes with many guest molecules, a reaction which can be used as a nucleic acid probe. In this paper, the most stable conformations of RhB-β-CDen and RhB-β-CD have been determined by fluorescence experiments and analyzed by molecular modeling simulation. The interaction between RhB-β-CDen and two guest molecules, 1-borneol and cyclohexanol, has also been investigated. The results showed that RhB-β-CDen has a stronger interaction with 1-borneol than with cyclohexanol. Borneol could push the three aromatic-rings of rhodamine B out of the CD cavity, while the cyclohexanol could not. The interactive sites of host and guest are also presented.

Key words: RhB-β-CDen, RhB-β-CD, recognition, host + guest, cyclohexanol, molecular dynamics, 1-borneol

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven, eight and more D-glucopyramose units (α-, β-, γ-CD, etc.), which play a role as typical hosts. β-CD is characterized by units of 1,4-glycosidic-linked β-D-glucopyranose units ($^4C_1$ chair conformation). CDs accommodate a variety of organic compounds in their central cavities in aqueous solution [1]. The sensitivity of the complex system with many kinds of guest molecules depends on van der Waals interaction, hydrophobic interaction and the polarity and bulk of the guest substances.

It is known that the fluorescence of RhB-β-CDen originates with the rhodamine B group [2, 3], which has a three-aromatic-ring group and a benzene ring. Both rhodamine B-ethylenediamine-β-cyclodextrins (RhB-β-CDen) and rhodamine B-β-cyclodextrins (RhB-β-CD) can probe DNA. This mechanism can be used to control a DNA sensory system like an on/off switch using host + guest recognition [4, 5]. According to experimental results [6, 7], it appears that RhB-β-CDen itself associates and exists as either a dimer or monomer in the absence of guests. In the presence of guests, the associated states are converted into a host + guest (1 : 1) complex, which can be demonstrated through the intensities of excitation and emis-

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sion peaks of RhB-β-CDen monomer associated with various guest molecules. Since the emission intensity of RhB-β-CDen may be affected by the presence of the guests, it may be expected that RhB-β-CDen can be used as a unique host + guest sensory systems. In this paper, the possible structures of RhB-β-CDen and RhB-β-CD were determined by fluorescence experiments and analyzed by molecular modeling. We have further studied two host+guest sensory systems of borneol + RhB-β-CDen and cyclohexanol + RhB-β-CDen both experimentally and theoretically.

2. Method

2.1. Experimental methods

The two host molecules, rhodamine B-ethylenediamine-β-cyclodextrin (RhB-β-CDen) and rhodamine B-β-cyclodextrin (RhB-β-CD), were synthesized in our laboratory [8]. Other reagents were all analytically purified. The maximum fluorescence intensity of RhB-β-CDen was recorded with a Shimadzu UV-240 spectrophotometer in the temperature range of 30–80° C.

2.2. Theoretical methods

Structures of RhB-β-CDen and RhB-β-CD were constructed using Biosym Software. Each structure was calculated by minimization and molecular dynamics for 1000 steps. The models of the two guest molecules were optimized in 300 steps. The parameters were those of the CFF91 forcefield. All calculations were performed on a Silicon Graphics workstation 4d310.

Possible interactive positions between RhB-β-CDen and 1-borneol were determined by docking calculation. The objective of a docking calculation is to evaluate the interaction energies of many orientations of one molecule relative to the other, while searching for the optimum orientations that results in low interaction energies. The docking energy in the CFF91 forcefield is computed as follows:

$$E_{\text{interaction}} = \sum_i \sum_j \left(\frac{A_{ij}}{R_{ij}^9} - \frac{B_{ij}}{R_{ij}^6} + \frac{q_i q_j}{\varepsilon r_{ij}} \right) \tag{1}$$

3. Results and Discussion

3.1. Structures of RHB-β-CDEN and RHB-β-CD

Figure 1 shows the intensity variation of host molecule RhB-β-CDen at the maximum fluorescence absorption. It can be observed that the maximum absorbance at 560 nm decreases slowly with increasing temperature. It is known that when

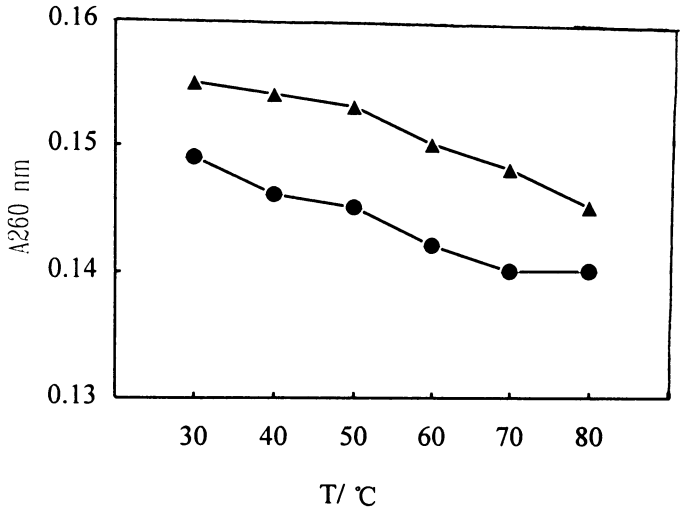

Figure 1. Temperature-dependence curve of absorbance at 560 nm. ●, without guest; ▲, with 1-borneol.

the RhB group is in a hydrophobic environment, such as an enzyme or micelle, its fluorescence will be sensitized [9]. Therefore, we can assume that at low temperatures the most probable conformation is where the RhB group inserts into the CD cavity. The CD cavity could provide a hydrophobic microenvironment for the RhB group, thus sensitizing the fluorescence of the RhB group. At higher temperatures, the RhB group obtained sufficient kinetic energy to release itself from the CD cavity into the aqueous environment. The fluorescence intensity of the RhB group decreases in an hydrophilic environment.

Molecular mechanics and dynamics simulations were used to determine the most stable structure of RhB-β-CDen and RhB-β-CD. It is obvious that the relative positions between the RhB group and the CD cavity have only three main possibilities; we therefore built three structural models of RhB-β-CDen and RhB-β-CD. The three minimized structural models of RhB-β-CDen are shown in Figure 2.

(a) one side of the three-aromatic-ring of rhodamine B inserts into the CD cavity (Figure 2a).
(b) the benzene ring of RhB and ethylenediamine are positioned in the CD cavity, but the three-aromatic-ring is outside the CD cavity (Figure 2b).
(c) the three-aromatic-ring of PhB and the benzene ring are both apparently outside the hydrophobic cavity of the cyclodextrin (Figure 2c).

According to the conformational energy summary, the order of the conformational stability is Figure 2a > 2b > 2c, as shown in Table I. The theoretical results are in agreement with the experimental assumption that the conformation of the RhB

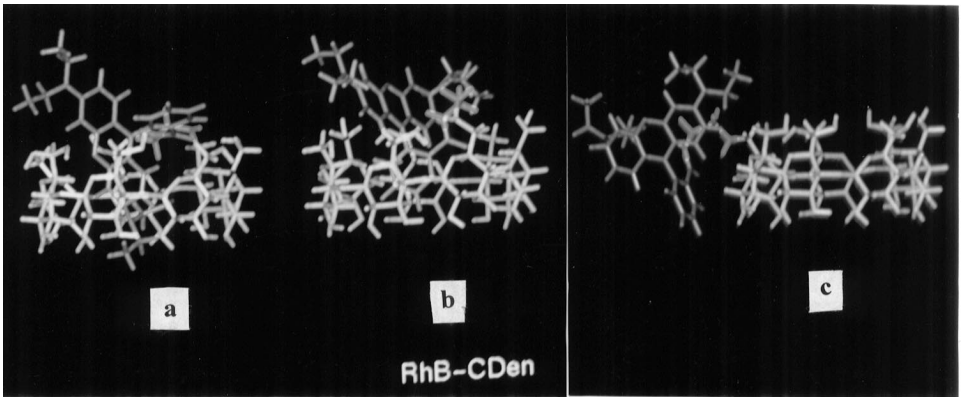

Figure 2. Three possible conformations of RhB-β-CDen.

Table I. Conformational energies of the three structures of RhBβ-CDen and RhB-β-CD (kcal/mol).

Host	Model	E_{total}	E_{bond}	E_θ	E_ϕ	E_{vdw}	E_{el}
RhB-β-CDen	Figure 2a	−28.5	18.1	78.5	−229.7	19.4	136.7
	Figure 2b	−22.7	18.5	77.0	−232.1	27.3	136.9
	Figure 2c	−6.4	17.9	72.7	−236.4	44.1	144.2
RhB-β-CD	Figure 3a	−0.2	16.4	71.0	−209.2	27.6	144.1
	Figure 3b	5.5	16.8	71.6	−212.5	35.5	145.4
	Figure 3c	14.4	16.7	70.8	−211.4	46.6	142.9

$$E_{\text{bond}} = \sum_r k(r - r_0)^2,\ E_\theta = \sum_\theta k_\theta(\theta - \theta_0)^2,\ E_\phi = \sum_\phi k_\phi(1 + \cos(n\phi - \tau)),$$

$$E_{\text{vdw}} = \sum_{i>j}\left[\frac{A_{ij}}{r_{ij}^9} - \frac{B_{ij}}{r_{ij}^6}\right],\ E_{\text{el}} = \sum_{i>j}\left[\frac{q_i q_j}{\varepsilon r_{ij}}\right].$$

group inserting into the CD cavity is the most stable one. The biggest difference of conformational energies occurred in the van der Waals energies, the other energies being very similar. It is shown in Table I that the lower the van der Waals energies, the lower are the total energies. In fact, different groups in the CD cavity are crucial to the stability improvement of the host structure of RhB-β-CDen.

The conformation stability of RhB-β-CD is similar to that of RhB-β-CDen. The mechanism of molecular capture in the three models of RhB-β-CD (Figure 3) is also the same as that of RhB-β-CDen, but it seems that the RhB group of RhB-β-CDen is more flexible than that of RhB-β-CD due to the —NH$(CH_2)_2$NH (ethylenediamine) group. Therefore the RhB group of RhB-β-CDen will release from the β-CD cavity more easily than that of RhB-β-CD. From the theoretical results, it is possible to conclude that the van der Waals interaction is important in forming stable structures of RhB-β-CDen and RhB-β-CD.

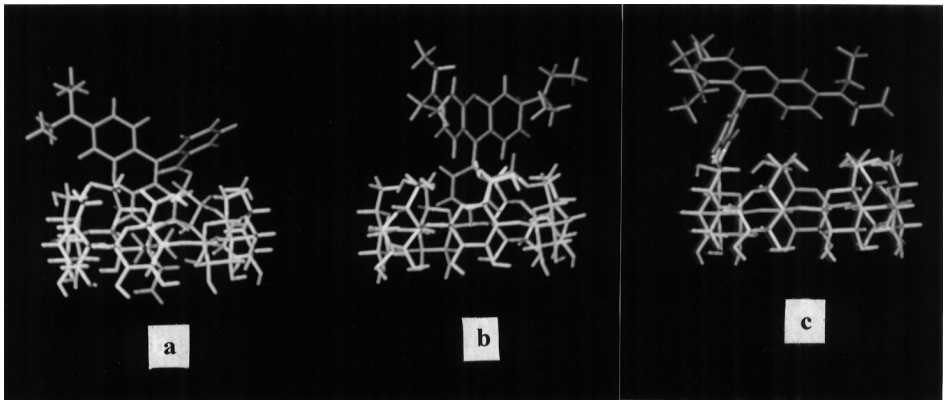

Figure 3. Three possible conformations of RhB-β-CD.

3.2. STRUCTURE OF HOST + GUEST RECOGNITION SYSTEM

We know that the most stable conformation of RhB-β-CDen at low temperatures is when the RhB group exists in the CD cavity. The fluorescence intensity of RhB-β-CDen is strong in the hydrophobic environment of the CD cavity. According to experiments by Ueno [7], a dansyl-modified β-cyclodextrin can detect many organic compounds with high sensitivity. 1-borneol was detected with higher sensitivity than cyclohexanol. When guest 1-borneol was added, the dansyl moiety moves from the interior of the hydrophobic cavity toward the bulk water environment, which leads to a reduction of fluorescence intensity. We have also studied the binding of the two guest molecules borneol and cyclohexanol to the host molecule RhB-β-CDen [6]. Binding constants ($\Delta I / I^0$) were measured for two different guests, where I^0 is the fluorescence intensity of the host molecule in the absence of guests, I is the fluorescence intensity of the host + guest compound in the presence of guests, and ΔI is $I^0 - I$. In the process of host + guest recognition, when the concentrations of RhB-β-CDen and guest molecule 1-borneol are 1×10^{-4} mol/L and 4.8×10^{-4} mol/L, respectively, the binding factor is 0.334. When the guest molecule is cyclohexanol, the binding factor is 0.102. Borneol is detected with much higher sensitivity and cyclohexanol is detected with substantially lower sensitivity, which further reveals that borneol is bound much more strongly to the CD cavity than cyclohexanol. Figure 1 also shows the fluorescence absorbance changes of RhB-β-CDen at 560 nm with increasing temperature in the presence of 1-borneol. The absorbances with 1-borneol are higher than those without, which indicates that 1-borneol inserts into the CD cavity and the RhB group is somewhat expelled from it. As the temperature increases more of the guest squeezes into the CD cavity and RhB groups are more easily released from the cavity. From our experimental results, we can conclude that the mechanism of RhB-β-CDen binding with guests is similar to that of the dansyl-modified β-cyclodextrin.

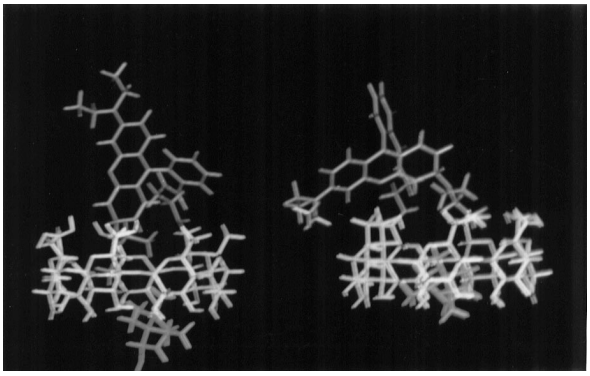

Figure 4. The starting and final structures of borneol + RhB-β-CDen system. Left: starting structure, right: final structure.

Table II. Interaction between host and guest of the two systems (kcal/mol).

Guest	Host	E_{total}	E_{vdw}	E_{el}
Borneol	RhB-β-CDen	−25.1	−22.1	−3.0
Cyclohexanol	RhB-β-CDen	−11.5	−9.1	−2.5

In the following theoretical section, the possible interactive sites between RhB-β-CDen and two guest molecules (borneol and cyclohexanol) have been determined by docking calculation.

The structure of RhB-β-CDen (Figure 2a) was selected as the host molecule. In fact, this structure is the three-aromatic-ring of rhodamine B inserting into the cavity of cyclodextrin. The two guest molecules are borneol and cyclohexanol, as in our experiments. The conformational energies of borneol and cyclohexanol are −9.6 and −23.4 kcal/mol, respectively.

Three possible interactive systems of host + guest were obtained by using the docking method, calculating 3500 steps. The system with the strongest interaction between host and guest was selected for calculation. The final structures of host + guest system were determined after molecular mechanics and dynamics calculations for 1000 steps, respectively. Figures 4 and 5 show the starting structures determined by docking and final structures after energy minimization. At the starting structures, the interactive orientation is obtained: borneol and cyclohexanol are all at the lower edge of cyclodextrin. For the final structures, borneol inserts into the cavity of cyclodextrin and the three-aromatic-ring is pushed completely out of the cavity, while cyclohexanol is a little distant from the cavity and the three-aromatic-ring inserts more deeply into the cavity. The interaction between borneol and β-CDen is stronger than that between cyclohexanol and β-CDen. The calculated results of two host + guest systems are shown in Tables II and III.

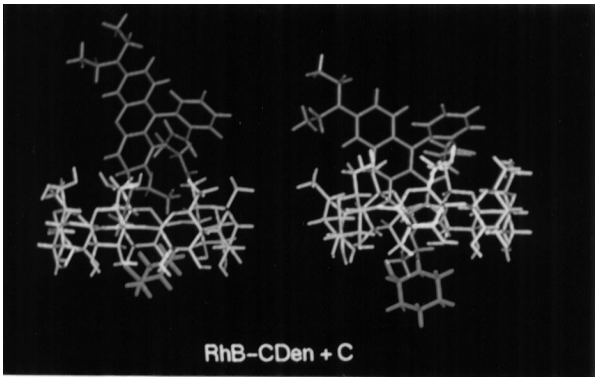

Figure 5. The starting and final structure of cyclohexanol + RhB-β-CDen system. Left: starting structure, right: final structure.

Table III. Intramolecular energies of two host + guest systems (kcal/mol).

System	Molecule	E_{total}	E_{bond}	E_{θ}	E_{ϕ}	E_{vdw}	E_{el}
Borneol	Borneol	−8.5	1.6	19.5	−23.0	10.9	−5.7
+RhB-β-CDen	RhB-β-CDen	−14.2	17.8	73.0	−234.8	37.9	140.7
Cyclohexanol	Cyclohexanol	−23.2	0.3	1.1	−20.7	3.8	−6.6
+RhB-β-CDen	RhB-β-CDen	−23.1	17.7	80.2	−232.5	19.6	142.5

From the calculated data (Table II), we can see that the interaction between borneol and RhB-β-CDen is more than twice as strong as that between cyclohexanol and RhB-β-CDen. The electrostatic energies of the two systems are very similar, but the van der Waals energy between borneol and RhB-β-CDen is much lower than that between cyclohexanol and RhB-β-CDen. van der Waals energy is the main contributor to the interaction of host + guest system. It seems that in these two systems the hydrophobic groups of guests are more important than hydrophilic ones, strengthening the interaction between host and guest.

In host + guest systems, the conformation energies of two guest molecules change little in comparison with those of the separated form, but those of the host in different systems change a great deal. For instance, the conformation energy of host RhB-β-CDen is −14.2 kcal/mol in the borneol + RhB-β-CDen system, and −23.1 kcal/mol in the cyclohexanol+RhB-β-CDen system, while the energy for separated RhB-β-CDen is −28.5 kcal/mol. Therefore in the former system the conformational energy of RhB-β-CDen increases by 14.3 kcal/mol, while in the latter one the energy increases by 5.4 kcal/mol. The major energy difference results from intramolecular van der Waals interaction. Thus guest molecules may change the relative positions of the seven oligosaccharide units of β-CDen. Since the interaction between borneol and RhB-β-CDen system is stronger, the conformation of

RhB-β-CDen greatly deforms to accommodate guest molecules, and then the host energy becomes higher.

The shape of the small guest molecule is also an important factor in influencing the interaction between host and guest [4]. Although both 1-borneol and cyclohexanol have one hydrophilic hydroxyl group, their shapes are different from each other. Borneol is a bicyclic compound and cyclohexanol a monocyclic one. The two cyclic groups in borneol could form a peaked moiety, which possibly means that 1-borneol inserts into the CD cavity move easily than cyclohexanol.

4. Conclusion

The most possible conformations of RhB-β-CDen and RhB-β-CD are one side of the three- aromatic-ring of rhodamine B inserting into the cavity of cyclodextrin, as determined by fluorescence experiments and further analyzed by molecular modeling simulation. Our experimental and theoretical results show that the binding ability of borneol to RhB-β-CDen is much stronger than that of cyclohexanol. The extent of the depression of the excimer emission was used as a measure of the sensitivity of host + guest system. Borneol was detected with higher sensitivity, and cyclohexanol was detected with substantially lower sensitivity. Borneol can insert into the CD cavity and push the three-aromatic-ring of rhodamine B out of the cavity towards the bulk water environment. While cyclohexanol is only at the edge of the cavity and could not insert into it, the cavity is still fully occupied by the three-aromatic-ring of rhodamine B. The van der Waals interaction between host and guest is the main contributor to the binding ability. The geometric shape of borneol and cyclohexanol is also an important factor that influences the binding ability. During the interaction process, the conformation of host molecule RhB-β-CDen changes to accommodate various guest molecules.

Acknowledgement

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