Nanotube Formation in Solution between β-Cyclodextrin and Cinchonine

Xianhong Wen, Ming Guo, Ziyang Liu,* and Fei Tan
Department of Chemistry, Zhejiang University, Hangzhou, 310027, P. R. China

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The nanotube could been formed between the drug cinchonine and β-cyclodextrin in solution when they were mixed together and sonicated for about 1 h. Fluorescence anisotropy methods characterized the number of cyclodextrin in nanotube. AM1 calculation implied that H bonds played an important role to stabilize the nanostructure.

Cyclodextrins (CD), containing 6(α-CD), 7(β-CD), or 8(γ-CD) D-glucose units, have high molecular recognition ability towards guest molecules because of their hydrophobic internal cavity and hydrophilic external surface. It has been extensively used to enhance the solubility, chemical stability, and bioavailability of drugs. 1:1 and 1:2 inclusion complexes have been extensively studied, though other stoichiometries can also be formed. Nanotubular structure is another important type of supramolecular assemblies in cyclodextrin chemistry. Recently, Li et al. found that β and γ-CD can form nanotubes by including rodlike moleculars, such as all-*trans*-1,6-diphenyl-1,3,5-hexatriene (DPH).[1] Agbaria and Gill have reported that other rodlike molecules PBD, PPO, PPD, BBOD can also form extended nanotubes with γ-CD at higher concentrations.[2] But such structure between drug and cyclodextrins has not been reported.

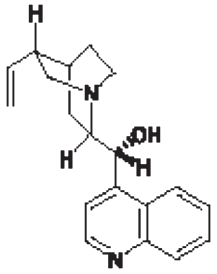

Figure 1. Structures of cinchonine.

Cinchonine (Cin) (**1**) is the primary drug of choice in the treatment of malri, and also exhibit K^+ channel blocking and antiarrhythmic activities.[3] But, it is poorly soluble in water. The inclusion behavior between cyclodextrins and cinchonine has been characterized to be 1:1 and 2:1 coexistent binding modes by 2D-NMR and mass spectrometry.[4,5] It is found that clear solutions of β-CD (10 mM) and Cin (10×10^{-3} M) will turn turbid if blended together and sonicated for several minutes. This phenomenon implies that some complexes in large size might exist.[6]

The stock solution of Cin(dissolved in methanol) and β-CD were mixed, methanol was evaporated completely by nitrogen bubbling, the mixture was sonicated for 1 h, and then incubated for one night before measurements.

The measurement of the steady-state fluorescence anisotropy provides a method of estimating the relative size of the Cin-β-CD nanotube according to Perrin-Weber formula:[7]

$$r_0/r = 1 + \tau RT/\eta V$$

where r_0 is the maximum value of anisotropy in the vitrified solution of glycerol, the measured value was 0.333. When the fluorescence lifetime τ and viscosity η remain constant, an increase in the fluorescence anisotropy suggests an increase in the size of the complex. In this way, the relative size of the Cin–β-CD complex could be estimated by means of the corresponding values of r.

$$r_2(r_0 - r_1)/r_1(r_0 - r_2) = V_2/V_1$$

where r_1 and r_2 are the values of the fluorescence anisotropy measured in two different systems, whereas V_1 and V_2 stand for the effective volumes of these two systems. However, the ratio V_1/V_2 can be approximated by the ratio of the molecular weights of the appropriate CD–Cin complexes, viz., M_1/M_2. The volume of 1:1Cin–β-CD complex was chosed to be V_1, so the value of V_2/V_1 was equal to number of β-CD molecules in the nanotube.[8]

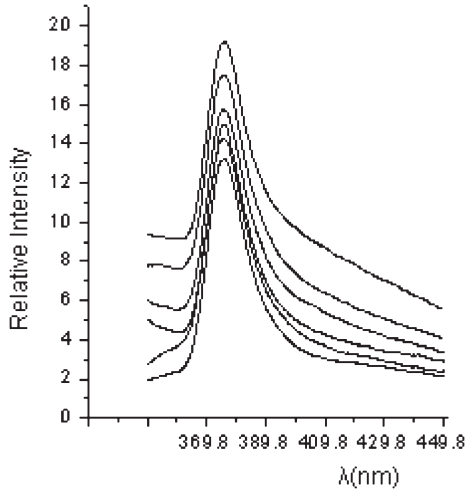

Figure 2. Fluorescence spectra of Cin(1×10^{-7} M) in β-CD solution. (from buttom to top, [β-CD] = 0, 1, 2, 4, 6, 10 mM)

When [Cin] $\leq 1 \times 10^{-7}$ M, the fluorescence intensity of complex can be presented by the Benesi-Hildebrand equation (Figure 2):

$$1/(F - F_0) = 1/(F - F_0) + 1/\alpha K C_{CD}$$

It implies that only 1:1 complex formed. The fluorescence anisotropy value(r1) of 1:1 complex was measured 0.051.

To estimate the number of β-CD molecules in the nanotube, the fluorescence anisotropy values (r_2) of Cin(2×10^{-5} M) at varying concentrations of β-CD were investigated (Figure 3). The plots show that with increasing the concentration of β-

CD, the fluorescence anisotropy of Cin and the number of β-CD first increase, and then reach a plateau at [β-CD] = 8 mM. This implies that most of the Cin in the solution can be included by β-CD at [β-CD] $\geq$ 8 mM. The maximum number of β-CD in the nanotube is estimated to be about 10 when the concentration of β-CD is above 8 mM.

Some physical or chemical conditions may have important influence on the formation of the nanotube. The fluorescence anisotropy decreases as the temperature increases, which means that the rotation of Cin molecule becomes fast and the nanotube become unstable at high temperature. Furthermore, we find that when the pH of a pure aqueous solution increases above 11, the number of β-CD in the nanotubes will decrease, when pH is above 13, the nanotubes can not form at all. In alkaline solution, the OH group of Cin is dissociated. Malliaris et al. considered that the secondary hydroxy groups of CD would been dissociated in solution (pH > 12) and the nanotube could not form.[9]

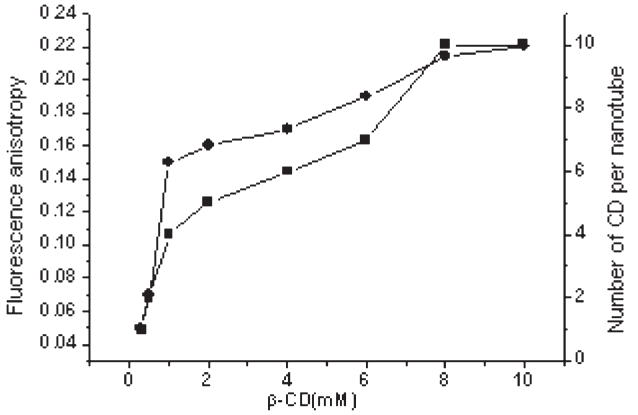

Figure 3. Steady-state fluorescence anisotropy (●) and average number of β-CD per nanotube vs [β-CD] (■).

To predict the structure of nanotube, AM1 calculation was performed to get the energy-minimum structure of basic nanounit (3β-CD + 2Cin) (Figure 4).[10] The results showed that the ethylene moiety of Cin entered fully into the cavity of one β-CD from narrow side, simultaneously, the naphthyl moiety of another Cin was included by the wider side of the same β-CD, cyclodextrin thus could include different Cin molecules from either sides. The long nanotube was assemblied by such a model. In 3β-CD + 2Cin complex structure, total eleven H bonds were formed between the OH group of the neighboring cylcodextrins, the OH group of Cin also formed two H bonds with the OH group of cyclodextrins. The driving force for cyclodextrin–guest binding was commonly attribute to hydrophobic interactions, hydrogen bonding, or van der Waals interaction in solution. Because the ethylene group and naphthyl moiety of Cin are hydrophobic, the two group can easily enter the hydrophobic cavity of cyclodextrin in aqueous medium. The AM1 calculation show that H bonds play an very importment role to stabilize the nanotube. Therefor, the nanotube will break down in alkaline solution.

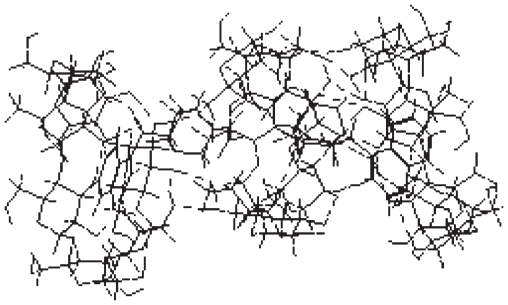

Figure 4. The energy-minimum structure of basic nanounit (3β-CD + 2Cin). (Hydrogen bonds are shown with dashed lines.)

In conclusion, drug with suitable group and dimension (such as cinchonine) can form nanotube with cyclodextrins. The use of cyclodextrins as the nanoparticale and nanocapsule system for drug delivery may be very interesting and practical in the future. Further studies for solid nanotube formed between drug–cyclodextrin are in progress in our laboratory.

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References and Notes

1 G. Li and L. B. Mcgown, *Science*, **246**, 249 (1994).
2 R. A. Agbaria and D. Gill, *J. Photochem. Photobiol., A*, **78**, 161 (1994).
3 S. Levy and S. Azoulay, *J. Cardiovascul. Electrophysiol.*, **5**, 635 (1994).
4 Y. Liu, L. Li, H. Y. Zhang, Z. Fan, and X. D. Guan, *Bioorg. Chem.*, **31**, 11 (2003).
5 X. H. Wen, Z. Y. Liu, and T. Q. Zhu, *Bioorg. Chem.*, accepted.
6 C. F. Zhang, X. H. Shen, and H. C. Gao, *Chem. Phys. Lett.*, **363**, 515 (2002).
7 R. M. Anderton and J. F. Kauffman, *J. Phys. Chem.*, **98**, 12117 (1994).
8 G. Pistolis and A. Malliaris, *J. Phys. Chem. B*, **102**, 1095 (1998).
9 G. Pistolis and A. Malliaris, *J. Phys. Chem.*, **100**, 15562 (1996).
10 All calculations were performed with Gaussian 98 soft (SGI64-G98RevA.11.2) on an SGI workstation with 8 parallel CPU and 2G memory. The initial structures of cinchonine and ß-cyclodextrin were constructed with the help of Chem3D and optimized with AM1 from the crystal structure. The initial complex structure was constructed by docking the cinchonine molecular into cyclodextrin cavity. All structures were minimized using a conjugate gradient optimization procedure until a root-mean-square (RMS) value of 0.01 kcal mol^{-1} $Å^{-1}$ was obtained.

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