Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 32 (2004) 223–233
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Evidence for the 2:1 molecular recognition and inclusion behaviour between β- and γ-cyclodextrins and cinchonine

Xianhong Wen,[a] Ziyang Liu,[a,*] Tianqiang Zhu,[a] Miaoqin Zhu,[b] Kezhi Jiang,[a] and Qiaoqiao Huang[a]

[a] *Department of Chemistry, Zhejiang University, Hangzhou 310027, PR China*
[b] *Department of Chemistry, Zhejiang Education Institute, Hangzhou 310027, PR China*

Available online 12 May 2004

Abstract

Cinchonine (Cin) is the primary drug of choice in the treatment of malaria, but its poor solubility has restricted its use via the oral route. Cyclodextrins (CDs) form inclusion complexes with cinchonine to form soluble complexes. This interaction was investigated by solubility studies, electrospray ionization mass spectrometry (ESI-MS), and molecular modeling. ESI-MS evaluated successfully the nature of the solution-phase inclusion complexes. The experimental results showed that not only 1:1, but also stable 2:1 inclusion complexes can be formed between CDs and Cin. Multi-component complexes of β-CD–Cin–β-CD (1:1:1), γ-CD–Cin–γ-CD (1:1:1), and β-CD–Cin–γ-CD (1:1:1) were found in equimolar β- and γ-CD mixtures with Cin. The formation of 2:1 and multi-component 1:1:1 non-covalent CD–Cin complexes indicates that β- and γ-CD are able to form sandwich-type inclusion complexes with Cin in high concentrations. The phase-solubility diagram showed non-linear type A_p profile, indicating that more than one cyclodextrin molecule is involved in the complexation of one guest molecule. Molecular modeling calculations have been carried out to rationalize the experimental findings and predict the lowest energy molecular structure of inclusion complex.

Keywords: Inclusion complexation; Cinchonine; Cyclodextrins; Mass spectrometry; Molecular modeling

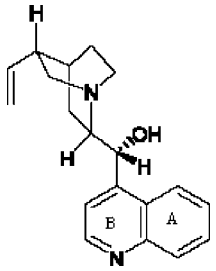
* Corresponding author. Fax: +86-571-8795-1895.
E-mail address: zyliu@css.zju.edu.cn (Z. Liu).

0045-2068/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2004.04.004

1. Introduction

The cinchona alkaloids such as quinine, quinidine, cinchonine, and cinchonidine have been used as effective antimalarial drugs [1]. Cinchonine also exhibit K^+ channel blocking and antiarrhythmic activities [2]. Other pharmacological effects of these alkaloids include reversal of multidrug resistance in different types of tumors [3]. In this activity, cinchonine (Scheme 1) has much lower toxicity and greater activity compared with other quinine related compounds [4]. Cinchonine also exhibits antihypertensive effects, possibly through blockade of α-adrenoceptor and Ca^{2+} channels. It is also a potent inhibitor of human platelet aggregation [5]. However, it is poorly soluble in water which limits its effectiveness in treating these conditions. Many compounds having molecular recognition capabilities enhance the solubility of the guest and help to understand numerous biological processes.

Cyclodextrins (CDs) (Scheme 2) are macrocyclic oligosaccharides, containing 6(α-CD), 7(β-CD), or 8(γ-CD) D-glucose units. As an important host compound, cyclodextrins have high molecular recognition ability towards guest molecules with suitable polarity and dimension because of their hydrophobic internal cavity and hydrophilic external surface [6]. This ability has been widely used in pharmaceutical applications. The pharmaceutical interest in CDs is due to their ability to enhance solubility, chemical stability, and bioavailability of poorly soluble drugs, and reduce toxicity and control the rate of release [6,7]. Cyclodextrins have also been used as chiral selectors for capillary electrophoresis enantiomer separations and analytical purposes [8]. Cyclodextrin usually forms 1:1 complexes with a guest molecule. When a guest molecule is bulky or long relative to the dimensions of CD cavity, two CD molecules are frequently bound to a single guest molecule to form a 2:1 CD–guest complex [9], The stoichiometric ratio of cyclodextrin and guest are usually characterized by fluorimetric, NMR, and mass spectrometry methods.



Scheme 1. Cinchonine.

Scheme 2. Cyclodextrins.

Electrospray ionization mass spectrometry (ESI-MS) has many advantages in the study of inclusion complexes between drug molecules and cyclodextrins including high sensitivity, speed, and a very low level of sample consumption. Electrospray ionization is one of the softest methods available to date [10]. ESI-MS allows detection and study of all kinds of different molecular associations between the guest and the host (such as enzyme–substrate, receptor–ligand, and protein–nucleic acid bindings). Strictly speaking, mass spectrometry only reflects the properties of gas-phase species. Nevertheless, the direct correlation between gas-phase complex and the solution behaviour is often good for ESI-MS [11,12]. Consequently, this method is used to analyze solution-phase molecular recognition and inclusion processes. Recently, Guo and others have successfully studied molecular complexes between various drugs (including diclofenac sodium, lorazepam, and the anti-inflammatory drug oleanolic acid) and cyclodextrins using ESI-MS [13–15]. The driving forces for the complex formation have been attributed to hydrophobic interactions, van der Waals interactions, hydrogen bonding, and release of ring strain in the CD cavity. Due to the limitations of the experimental methods, molecular modeling is frequently used to rationalize experimental findings concerning molecular and chiral recognition by cyclodextrins. Molecular modeling methods are valuable tools for detailed information on the geometry and the interaction energy of the inclusion compounds. Most computations for CD employ semiempirical AM1 or PM3 methods due to its large size [16,17].

In previous work [18], it was concluded that there were two 1:1 binding models between β- or γ-cyclodextrin and cinchonine based on the fluorimetric and NMR measurements. However, we have now obtained direct evidence in ESI-MS experiment for not only 1:1, but also 2:1 binding models of CDs and cinchonine. In addition, we have carried out solubility measurements and molecular modeling of these complexes to fully characterize the binding behavior between cyclodextrin and cinchonine. The work provide evidence for the formation of a 2:1 binding model between cyclodextrin and cinchonine.

2. Experimental

2.1. Materials

β- and γ-cyclodextrin (99.5%) were purchased from Sigma Chemical and purified by recrystallization from distilled water. Cinchonine was commercially available from Aldrich Chemical. Standard solutions of β- and γ-CD with concentrations of 0.01 M were prepared in deionized water. In addition, a 0.01 M cinchonine solution was prepared in methanol.

2.2. Solubility measurement

The phase-solubility diagram was determinated according to the Higuchi and Connors method [19]. Aqueous solutions of β-CD or γ-CD with concentrations of 0, 1.0, 2.0, 3.0, 4.0 and 5.0×10^{-4} M were prepared. Excess amounts of cinchonine were

added to each solution of CD. The solutions were sealed and shaken at ambient temperature for 48 h, followed by an equilibrium period (7 days), then centrifuged and filtered. Their absorption was measured by SPECORD 2000(752 type) UV spectrophotometry at 285 nm. The presence of CDs did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate.

2.3. Mass spectrometry

ESI-MS experiments were performed using a LCQ DECA XP ion trap mass spectrometer (ThermoFinnigan) equipped with an electrospray source. The sample was introduced via a syringe pump at a flow rate of 3 μL/min. High flow rate nitrogen gas was employed as the nebulizing gas as well as the drying gas to aid desolvation. The sheath gas flow rate was 0.5 L min^{-1}. After optimization of the MS parameters, the spray voltage was set to 5.0 kV in the positive mode, and the heated metal capillary temperature was set at 275 °C. The capillary voltage and tube lens offset were set at 15 and 40 V, respectively. The mass scale was calibrated by using the standard calibration procedure and compounds provided by the manufacturer.

2.4. Molecular modeling

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 β-cyclodextrin were constructed with the help of Chem3D and optimized with AM1 from the crystal structure. Modeling calculations were performed in two steps [20]. In the first step, for one β-cyclodextrin and one Cin, two inclusion models were assumed, i.e., the naphthyl (model 1), or the ethylene one, and were stepwise inserted into the cavity of CD. Subsequently, the geometry of the host–guest complex was completely optimized by AM1 without any restriction in order to determine the minimum-energy geometry. In the second step, for two β-cyclodextrin and one Cin, the optimized β-CD–Cin complex was allowed to dock into the second β-CD from the ethylene group side or naphthyl side. Subsequently, the structure of 2:1 host–guest complex was completely optimized to determine the minimum-energy structure. 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.

3. Results

3.1. Solubility measurement

One of the most important applications of cyclodextrins in the pharmaceutical field is to enhance the aqueous solubility of drugs through complexation. The phase-solubility diagram, i.e., plots of solubility of guest as a function of cyclodextrin concentration, of the complex formation between Cinchonine and CDs are presented in Fig. 1. According to the Higuchi and Connors classification, the diagrams obtained

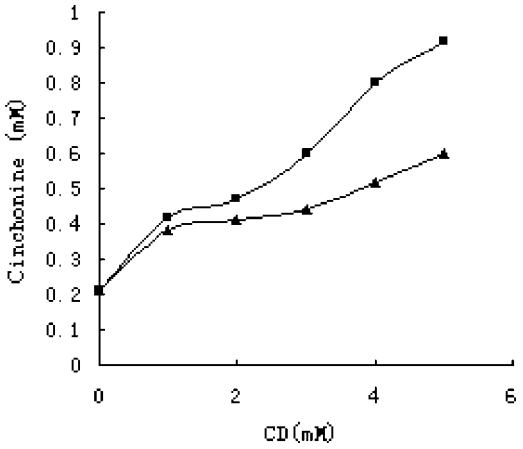


Fig. 1. Phase-solubility diagrams for cinchonine in the presence of β-CD (■), γ-CD (▲) in water at 25 °C.

were of A_p type (a soluble complex is formed), where the guest solubility of this type increases linearly with cyclodextrin concentration having a positive deviation from the straight line. From this curve, it can be seen that the apparent solubility of Cin increases due to the formation of a soluble inclusion complex between Cin and CDs. Dissolution of Cin from the β- and γ-CD complexes was three and fivefold greater, respectively, than that of the pure drug in water. The A_P type indicated higher order complex formation in which more than one cyclodextrin molecules are involved in the complexation [6,13,19].

3.2. Mass spectrometry characterization

Mass spectrometry is a powerful analytical tool, which provides unique selectivity, improved sensitivity, and valuable structural information. The use of ESI-MS for characterizing the stoichiometry and strength of interactions between synthetic or biological hosts and guests is a growing area of research [21]. In this experiment, the same volume of the host and guest solutions were mixed and shaken on a microvibrator (3500 spin/min) for 5 min to mix effectively before being introduced to the mass spectrometer. Preliminary experiments on solutions of CD–Cin were performed to determine the optimal electrospray ionization parameters. The analytes were found to give greater response in the positive mode. Therefore, the optimal electrospray ionization parameters and positive mode were selected for MS analyses. To eliminate the non-specific complexes, the heated metal capillary temperature was changed from 150 to 275 °C, but the relative abundance of complex was not diminished in high temperature up to 275 °C, so the experiment capillary temperature was set at 275 °C.

Fig. 2 illustrates the mass spectra of equimolar CD and Cin mixture. From the Fig. 2A, the peaks observed at *m/z* 295 and 1157, correspond to the singly charged $[Cin + H]^+$ and $[\beta\text{-CD} + Na]^+$, respectively. The most abundant peaks at *m/z* 1429 and *m/z* 2563 correspond to $[Cin + \beta\text{-CD} + H]^+$ and $[Cin + 2\beta\text{-CD} + H]^+$ which might be a

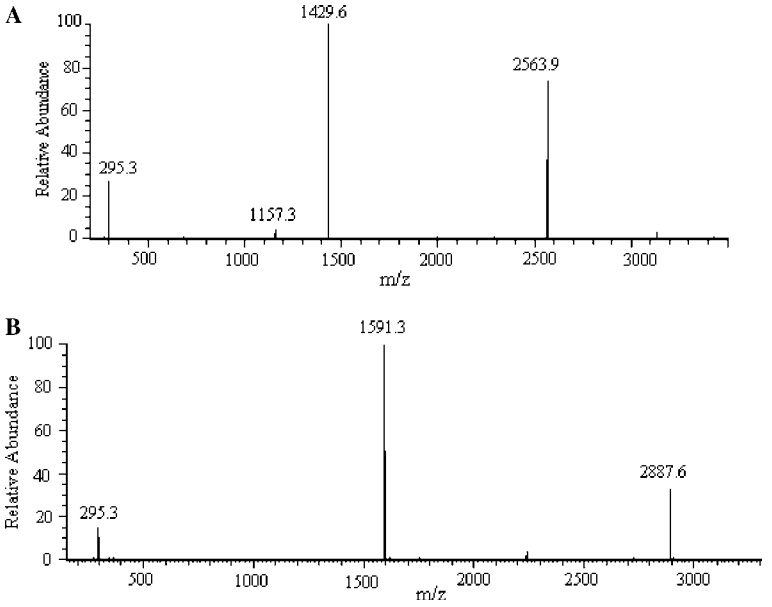


Fig. 2. ESI mass spectra of CD–Cin complexes: (A) β-CD with Cin (1:1 equimolar mixture) and (B) γ-CD with Cin (1:1 equimolar mixture).

sandwich-type complex. In Fig. 2B, a similar result is seen: the most abundant ion is at *m/z* 1591, corresponding to $[\gamma\text{-CD} + \text{Cin} + \text{H}]^+$, and the next peak is at *m/z* 2887, which corresponds to $[2\gamma\text{-CD} + \text{Cin} + \text{H}]^+$. The experimental results show that not only the 1:1 CD/Cin complex, but also the single charged state of the ions corresponding to 2:1 CD–Cin complexes are all found for β- and γ-CD in the positive ion mode. The 2:1 CD–Cin complexes between β- and γ-CD and Cin suggest that the ethylene and naphthyl group may be simultaneously included in the CDs'cavity.

To provide a reasonable estimate of the relative gas-phase stabilities of the complexes between the two CDs and Cin, we performed different mass spectrometry experiments where the two CD species were mixed in equimolar amounts with Cin under identical experimental conditions. The final concentration of β-CD, γ-CD, and cinchonine was 3.3 mM.The ESI mass spectra of the two kinds of CDs mixed together with Cin in equimolar amounts are shown in Fig. 3. There are mainly five peaks, at *m/z* 295, *m/z* 1429, *m/z* 1591, *m/z* 2563, and *m/z* 2887, corresponding to $[\text{Cin} + \text{H}]^+$, $[\text{Cin} + \beta\text{-CD} + \text{H}]^+$, $[\gamma\text{-CD} + \text{Cin} + \text{H}]^+$, $[\text{Cin} + 2\beta\text{-CD} + \text{H}]^+$, and $[2\gamma\text{-CD} + \text{Cin} + \text{H}]^+$, respectively. Also, a relative high abundance at *m/z* 2725 was observed, which corresponded to $[\beta\text{-CD} + \gamma\text{-CD} + \text{H}]^+$. No cluster ions of CDs were observed in the mass spectrum, which implies that the 2:1 CD–Cin complexes between β- and γ-CD and Cin were sandwich-type complexes (β-CD–Cin–γ-CD), and not β-CD–γ-CD–Cin non-special aggregation complexes [13,22]. The relative intensities for 1:1 and 2:1 β-CD–Cin complexes were greater than those of γ-CD–Cin complex. The intensity decreases with an increase in the mass of the CD, as expected.

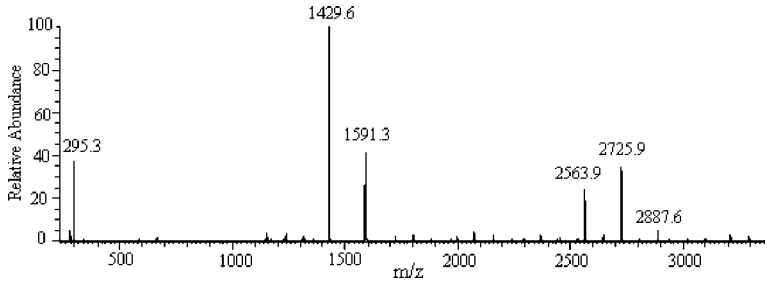


Fig. 3. ESI mass spectra of an equimolar mixture of β-CD, γ-CD with equimolar Cin.

This is primarily due to the fact that the energy required for the phase change of a gaseous ion from a liquid sample will typically increase with increasing mass within a series of like molecules.

To study the effect of sample concentration on the complex, the mass spectrometry experiments were carried out where the stock solution concentrations of cyclodextrin and cinchonine were diluted 10- and 50-fold. The final concentrations of cyclodextrin and cinchonine were 5×10^{-4} M in Fig. 4A. In Fig. 4B, they were 1×10^{-4} M. From Figs. 4A and B, the 1:1 and 2:1 CD–Cin complexes were clearly observed although the concentrations of CD and Cin were diluted. The signal to noise ratio has decreased. The 2:1 complex didn't disappear more rapidly than the 1:1 complex upon dilution. Hence, we considered that the 2:1 complex is a specific complex and not an experimental artifact.

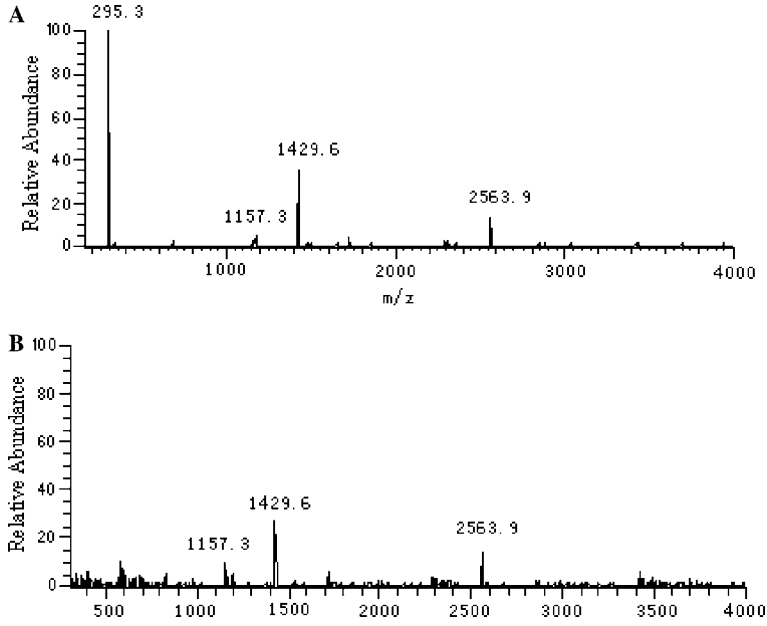


Fig. 4. ESI mass spectra of CD–Cin complexes. (A) 10 times diluted and (B) 50 times diluted.

3.3. Molecular modeling

Computational studies on host–guest interactions were carried out to find the most probable conformation of the complex and the appropriate three-dimensional representation of the complex. The energy minimum structures of β-CD–Cin complex calculated by Gaussian98 are showed in Fig. 5. Stabilization energy (ΔE) upon complexation was calculated for the minimum energy structure according to Eq. (1) [23,24]:

$$\Delta E = E_{\text{complex}} - (E_{\text{host}} + E_{\text{guest}}).$$

From Table 1, it can be seen that the complexation of Cin with β-CD through the ethylene moiety is less favorable than that with β-CD through the naphthyl moiety by an energy difference of 12.1 kJ/mol. In Fig. 5A, the ethylene moiety of Cin entered

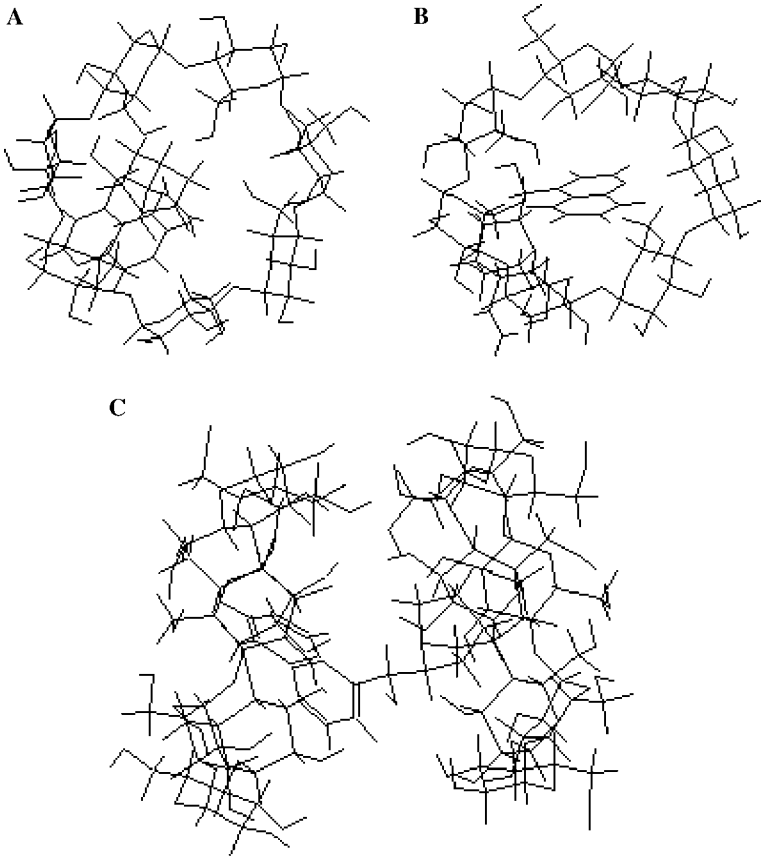


Fig. 5. Energy-minimized structure obtained by AM1 calculations for the β-CD–Cin complexes. (A) the ethylene moiety of Cin entering into CD, (B) the naphthyl moiety of Cin entering into CD, and (C) two CD included one Cin simultaneously.

Table 1
The interaction energy between β-cyclodextrins and cinchonine calculated with AM1

Species	CD:Cin	$E_{complex}$ (kJ/mol)	$E_{host} + E_{guest}$ (kJ/mol)	ΔE (kJ/mol)
β-CD–Cin (naphthyl side)	1:1	−6849.5	−6819.3	−30.2
β-CD–Cin (ethylene side)	1:1	−6837.4	−6819.3	−18.1
β-CD–Cin (two sides)	2:1	−13912.9	−13765.9	−147.0

fully into cavity of CD so that the hydroxy group of Cin was close to the wider ring of CD, and the distance of O–H···O (of cyclodextrin) was 2.17 Å. Similarly, in Fig. 5B, the ring A of naphthyl moiety was fully included by cyclodextrin, but ring B was only buried partly and the nearest distance of O–H···O (of cyclodextrin) was 2.16 Å. Because the distance is within 2.2 Å, intermolecular hydrogen bonds are formed between Cin and cyclodextrin [25,26]. For the energy-minimized structure show in Fig. 5C, not only one hydrogen bond exists between the hydroxy group of Cin and cyclodextrin, but two hydrogen bonds between two CDs are formed. Aromatic ring A and ethylene moiety were fully included by two CDs. However, the aromatic ring B containing the heteroatom only partly entered cavity and the formation of the 2:1 β-CD–Cin complex was the most energetically favorable of the three complexes. The driving forces for the complex formation could been attributed to these hydrogen bonds and hydrophobic interaction between non-polar parts of cinchonine (such as aromatic ring A and ethylene moiety) and cyclodextrins.

4. Discussion

In previous work, Liu et al. [18] characterized the complex between cyclodextrins and cinchonine using NMR and fluorimetry method. Molecular modeling calculations have now been carried out to rationalize the experimental findings. In a ^{1}H-NOESY experiment, cross-peaks were observed between protons in the ethylene group of ring A and cyclodextrins. These observations were explained by the coexistence of two different 1:1 binding modes. On the basis of molecule modeling, we find that the ^{1}H-NOESY spectrum could also be explained by a 2:1 CD–guest molecular complex. Although fluorescence spectral titrations gave evidence of a 1:1 stoichiometric

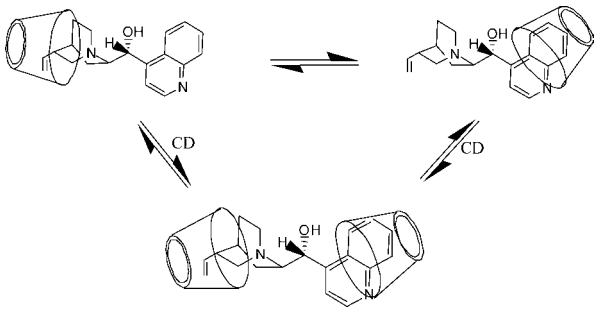


Fig. 6. Three binding models of cyclodextrins with cinchonine.

ratio between CD and Cin, it may not be true. In a 2:1 CD–guest complex, an unprotonated nitrogen heteroatom of the guest entered the interior cavity of cyclodextrin, which would quench the fluorescence, but the other less polar part of the guest molecule in the hydrophobic internal cavity may cause enhancement of fluorescence signal. As a result, the total fluorescence intensity may be very complicated. Under these conditions, the fluorimetric spectral titration methods to ascertain the stoichiometric rate are no longer reliable. If guest binds to cyclodextrin only in a 1:1 model, the phase-solubility curve would be the linear A_l type. In contrast, our experiment result gave non-linear A_p type curve. Furthermore, the mass spectrometry gave direct evidence for the 2:1 binding model. For these reasons, it is concluded that not only 1:1 but also a 2:1 cyclodextrin and cinchonine complex exist in solution. The models for cyclodextrins and cinchonine are showed in Fig. 6.

Acknowledgments

This work was supported by the National Natural Science Foundation of China and Specialized Research Fund for the Doctoral Program of Higher Education.

References

[1] J.W. Tracy, J.L. Webster, Drugs used in the chemotherapy of protozoal infections, in: J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, A.G. Gilman (Eds.), The Pharmacological Basis of Therapeutics, ninth ed., The McGraw Hill, New York, 1996, pp. 800–808.
[2] S. Levy, S. Azoulay, J. Cardiovasc. Electrophysiol. 5 (1994) 635–636.
[3] P. Genne, O. Duchamp, E. Solary, D. Pinard, J.P. Belon, Leukemia 8 (1994) 160–164.
[4] P. Genne, O. Ducham, E. Solary, J. Magnette, J.P. Belon, Cancer Drug Des. 10 (1995) 103–118.
[5] B.H. Shah, Z. Nawaz, S.S. Virani, I.Q. Ali, S.A. Saeed, A.H. Gilani, Biochem. Pharmacol. 56 (1998) 955–960.
[6] K. Uekama, F. Hirayama, T. Irie, Chem. Rev. 98 (1998) 2045–2076.
[7] P. Caliceti, S. Salmaso, A. Semenzato, T. Carofiglio, R. Fornasier, M. Fermeglia, M. Ferrone, Bioconjug. Chem. 14 (2003) 899–908.
[8] J.B. Chao, J.S. Li, D.P. Meng, S.P. Huang, Spectrochim. Acta A 59 (2003) 705–711.
[9] P. He, H.J. Lu, Y.L. Guo, Anal. Lett. 6 (2003) 491–508.
[10] C.A. Schalley, Int. J. Mass Spectrom. 194 (2000) 11–39.
[11] V. Gabelica, N. Galic, H. Claude, F. Rosu, C. Houssier, E.D. Pauw, J. Mass Spectrom. 38 (2003) 491–501.
[12] V. Gabelica, N. Galic, E.D. Pauw, J. Am. Soc. Mass Spectrom. 13 (2002) 946–953.
[13] M.Q. Guo, S.Q. Zhang, F.R. Song, D.W. Wang, Z.Q. Liu, S.Y. Liu, J. Mass Spectrom. 38 (2003) 723–731.
[14] Y. Cai, M.A. Tarr, G.X. Xu, T. Yalcin, R.B. Cole, J. Am. Soc. Mass Spectrom. 14 (2003) 449–459.
[15] (a) R. Kobetic, B.S. Jursic, S. Bonnette, J.S. Tsaia, S.J. Salvatorea, Tetrahedron Lett. 42 (2001) 6077–6082;
(b) J.B. Cunniff, P. Vouros, J. Am. Soc. Mass Spectrom. 6 (1995) 437–447.
[16] N.B. Boukamel, A. Krallafa, D. Bormann, L. Caron, M. Canipelle, S. Tilloy, J. Incl. Phenom. Macro. 42 (2002) 269–274.
[17] K.S. Song, C.R. Hou, L. Liu, X.S. Li, Q.X. Guo, J. Photochem. Photobiol. A: Chemistry 139 (2001) 105–109.
[18] Y. Liu, L. Li, H.Y. Zhang, Z. Fan, X.D. Guan, Bioorg. Chem. 31 (2003) 11–23.

[19] T. Higuchi, A.K. Connors, Advances in Analytical Chemistry and Instrumentation, in: C.N. Reilley, Interscience, New York, 1965, pp. 117–212.
[20] (a) T.W. Mu, L. Liu, X.S. Li, Q.X. Guo, J. Phys. Org. Chem. 14 (2001) 559–565;
(b) L. Liu, K.S. Song, X.S. Li, Q.X. Guo, J. Incl. Phenom. Macro. 40 (2001) 35–39.
[21] Y. Dotsikas, Y.L. Loukas, J. Am. Soc. Mass Spectrom. 14 (2003) 1123–1129.
[22] R. Arakawa, T. Yamaguchi, A. Takahashi, J. Am. Soc. Mass Spectrom. 14 (2003) 1116–1122.
[23] G. Piel, G. Dive, B. Evrard, Eur. J. Pharm. Sci. 13 (2001) 271–279.
[24] J. Taraszewska, K. Migut, M. Kozblio, J. Phys. Org. Chem. 16 (2003) 121–126.
[25] E.B. Starikov, W. Saenger, T. Steiner, Carbohydr. Res. 307 (1998) 343–346.
[26] G.A. Jeffrey, W. Saenger, Hydrogen Bonding in Biological Structures, Springer, Berlin, 1991.