J Incl Phenom Macrocycl Chem (2012) 72:413–421
DOI 10.1007/s10847-011-0005-8

ORIGINAL ARTICLE

A comparative study of complexation of enalapril with α-, β- and γ-cyclodextrins in aqueous medium: structure elucidation of inclusion complexes using NMR spectroscopic and molecular mechanics methods

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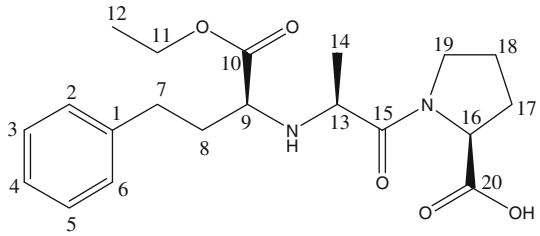
Received: 13 February 2011 / Accepted: 18 June 2011 / Published online: 8 July 2011

Abstract Structural studies of complexes of enalapril maleate with α-, β- and γ-cyclodextrins were carried by NMR spectroscopy and computational methods. The formation of complexes of enalapril with all the three cyclodextrins was established by chemical shift changes observed in the cavity protons of cyclodextrins in the presence of enalapril maleate. The stoichiometry of the complexes was determined to be 1:1 by ^{1}H NMR titrations studies using Scott's method. Intermolecular cross peaks observed in the 2D ROESY spectra of mixtures of enalapril maleate with three cyclodextrins helped in establishing the probable structures of these inclusion complexes which were supported by molecular mechanics (MM2) studies. Enalapril forms 1:1 inclusion complex with all the studied cyclodextrins through aromatic ring. The mode of approach of aromatic ring to the α-cyclodextrin cavity was found to be different from those of β- and γ-cyclodextrins, which were identical.

Keywords Cyclodextrin · Enalapril maleate · Inclusion complex · Molecular mechanics · ROESY

Introduction

Enalapril maleate (ENA) is a drug of choice, for adults and children, to withstand congestive heart failure, essential and renovascular hypertension, the most prevalent causes of mortality today [1]. It is an inhibitor of angiotensin converting enzyme which converts angiotensin I to angiotensin II which is responsible for narrowing the arteries and consequently leads to hypertension [2]. Its therapeutic applications have certain limitations due to poor aqueous stability, unpleasant taste and certain side effects [3]. The formation of maleate salt helps in stabilizing the drug to some extent but problem of storing the drug persists [4]. Attempts have been made to mask the bitter taste of the drug by forming sugar containing or sugar free liquid oral suspensions but this undertaking also lacks durability [5].



Enalapril

Cyclodextrins have been used extensively as pharmaceutical excipients to increase the solubility of poorly water soluble drugs, stability and bioavailability of drugs and to convert liquid drugs into microcrystalline powders, prevent drug–drug or drug–additive interactions, reduce gastrointestinal or ocular irritation, and reduce or eliminate unpleasant taste and smell in many pharmaceutical preparations [6–8]. Moreover, CD-inclusion complexes have proved to be an excellent model for studying the nature of noncovalent interactions in aqueous medium [9]. They have provided valuable insights concerning hydrophobic effect and London dispersion forces [10] and are good models for understanding the specificity of enzyme-substrate interactions [11]. The possible factors and various molecular forces, which may play a role in cyclodextrin complexation, have been widely investigated and discussed

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[9, 10]. However, the relative contributions and even the specific nature of the different forces involved in the process are still not well known [12].

Recently, there has been a great interest in the studies related to structure determination of drug-CD inclusion complexes since pharmaceutical use of CDs, in drug protection and targeting, now legally requires structural characterization of inclusion complexes. Of all the techniques, NMR spectroscopy is the most powerful method for these types of studies [13–15]. The most apparent motivation to use NMR for the study of these complexes is the interest to comprehend the driving forces and binding modes in these host–guest interactions, which relies on the ^{1}H NMR spectroscopic chemical shift values due to complexation. The spatial interactions between the hydrogen atoms of the host and guest molecules and corresponding three-dimensional geometry can easily be deduced by ROESY spectral data [14, 15].

We describe here the studies aimed at structure determination of inclusion complexes of enalapril with α-, β- and γ-CDs in aqueous medium by NMR spectroscopic and computational methods. We studied the complexation of enalapril maleate with β-CD earlier [16] and reported the formation of a 1:2 [β-CD:ENA] complex. Later, Longhi and coworkers [17] reported the formation of 1:1 [β-CD-ENA] complex which was supported by molecular mechanics studies. We, therefore, decided to reinvestigate the structure of β-CD-ENA inclusion complex and to compare the complexation processes of enalapril maleate with α-, β- and γ-CD since complexation of β-CD has been studies in aqueous medium earlier we avoided the use of buffer.

Experimental

Enalapril maleate was gifted by Nebula Healthcare, India, while cyclodextrins were obtained from Geertrui Haest, Cerestar Application Centre, Food & Pharma Specialities, France, and these were used without further purification. All the ^{1}H NMR and 2D NMR (COSY and ROESY) spectra were recorded on a JEOL 500 MHz instrument in D_2O at room temperature. No external indicator was used and HDO signal at 4.800 ppm has been used as internal reference throughout this work. The Chemical shift values (δ) are reported in ppm. NMR samples were prepared by taking calculated amounts of CD and drug in 0.5 ml of D_2O. The mixtures were then shaken or/and sonicated till clear solutions were obtained. The ENA/CD molar ratios were further confirmed by ^{1}H NMR spectral data.

^{1}H NMR spectra of pure α-, β-, γ-CDs and ENA as well as three sets of ^{1}H NMR spectra, viz. six spectra of each set of α-CD/ENA, β-CD/ENA and γ-CD/ENA mixtures having molar ratios (1:0.25, 1:0.5, 1:0.75, 1:1, 1:1.5 and 1:2), were recorded. The NMR samples of α-CD/ENA mixtures were prepared by taking fixed amount of ENA (4 mg) and calculated amounts of α-CD while those of β-CD/ENA and γ-CD/ENA mixtures were prepared by taking fixed amount of CD (9 mg) and calculated amounts of ENA. For the purpose of unambiguous assignments of ^{1}H NMR signals, ^{1}H-^{1}H COSY spectra were recorded for mixtures of ENA with three CDs. 2D ROESY spectra of (1:1) mixtures of ENA with three CDs were also obtained with a mixing time of 500 ms under spin lock conditions. Distinct peaks for bound and free form of ENA or CD were not observed in any of the spectrum, indicating a rapid exchange of guest between free and complexed state on the NMR time scale.

Results and discussion

^{1}H NMR spectra

The assignment of proton resonances of free forms of α-, β-, γ-CDs and ENA were found in good agreement with the reported assignments [6–8, 10, 13]. Different patterns of chemical shift changes for cavity protons of α, β- and γ-CDs, upon complexation with an aromatic ligand, are observed. Large upfield shift changes for H-5′ (dominant) and H-3′ (subordinate) are observed for β- and γ-CDs upon inclusion of aromatic ligands from wider rim side, but due smaller inner diameter of α-CD the guest only partially enters into the cavity resulting in upfield shift of H-3′ only [18]. In case of penetration of the guest into the CD cavity from narrower rim side, different patterns are observed [19].

A cursory examination of ^{1}H NMR spectra of the mixtures of ENA with α-, β- and γ-CDs revealed prominent upfield shift changes, compared to pure CDs, in both the cavity protons in the case of β- and γ-CD mixtures while only H-3′ proton signal of α-CD was perturbed in the presence of ENA. All the remaining CD protons showed only trivial shift changes except H-6′ which shifted significantly highfield in the case of γ-CD. These observations clearly support that ENA forms inclusion complexes with all the three CDs studied.

The chemical shift change ($\Delta\delta$) observed in the signal of H-3′ of α-CD in the presence of ENA (H/G = 1:1) was 0.0521 (Fig. 1a). The upfield shifts observed in the cavity protons of β-CD and γ-CD in the presence of ENA (H/G = 1:2) were 0.0541 (H-3′), 0.1256 (H-5′) (Fig. 1b) and 0.0390 (H-3′) 0.0483 (H-5′) (Fig. 1c), respectively.

Stoichiometry and binding constant

Several NMR versions [20–22] of Benesi-Hildebrand equation [23] are used for determining the stoichiometry

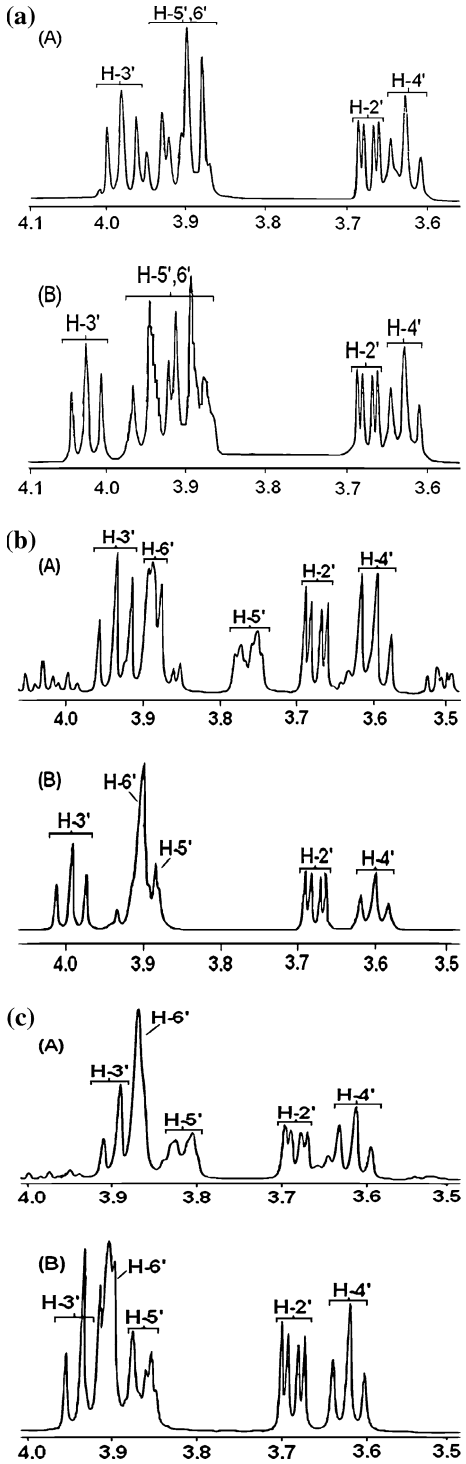


Fig. 1 Partial 500 MHz ^{1}H NMR Spectra of CDs as well as mixtures of CDs and enalapril maleate showing CD region: (**a**) (A) α-CD/ENA mixture (B) α-CD; (**b**) (A) *β*-CD/ENA mixture (B) *β*-CD; (**c**) (A) *γ*-CD/ENA mixture; (B) *γ*-CD

and binding constant of the inclusion complex. We have used Scott's method to determine the stoichiometry and binding constants of the complexes. Scott's [24] equation for a 1:1 drug-CD complex can be written as follows:

$$[\mathrm{CD}]/\Delta\delta_{\mathrm{obs}} = [\mathrm{CD}]/\Delta\delta_{\mathrm{c}} + 1/K_a \cdot \Delta\delta_{\mathrm{c}}$$

where [CD] is the molar concentration of the CD; $\Delta\delta_{\mathrm{obs}}$ is observed difference in chemical shifts for a given [CD] with varying concentrations of drug; $\Delta\delta_{\mathrm{c}}$ is the chemical shift difference between pure sample of complex and the free component at the saturation. A linear plot of $[\mathrm{CD}]/\Delta\delta_{\mathrm{obs}}$ vs. [CD] confirms the 1:1 stoichiometry of the complex with slope $1/\Delta\delta_{\mathrm{c}}$ and the intercept with the vertical axis to $1/K_a\ \Delta\delta_{\mathrm{c}}$ allowing the calculation of binding constant (K_a).

The chemical shift change data was obtained for ENA by varying concentration of α-CD while those for *β*-CD and *γ*-CD was obtained by varying concentrations of ENA. The data so obtained from NMR titrations was plotted in the form of [α-CD]/Δδ vs. [α-CD] for α-CD-ENA complex (Fig. 2a), [ENA]/Δδ vs. [ENA] for the complexes of *β*-CD (Fig. 2b) and *γ*-CD (Fig. 2c), with ENA, respectively, which gave excellent linear fits in all the three cases confirming that ENA forms 1:1 inclusion complexes with all the three CDs studied. The overall binding constant (K_a) were readily obtained which were found to be 116.2, 74.3, 199.2 M^{-1} for ENA complexes with α-, *β*- and *γ*-CD, respectively.

ROESY spectral studies

ROESY spectra of mixtures of enalapril maleate with three CDs were recorded to have an insight into the conformation of complexes formed. ROESY spectrum of α-CD-ENA mixture showed intermolecular cross peak only between H-3′ cavity proton with H-2,4,6 of ENA (Fig. 3a) suggesting that aromatic ring has partially entered the cavity and is positioned close to the wider rim.

Aromatic protons of enalapril showed close contacts with both the cavity protons, i.e. H-3′ and H-5′, in the ROESY spectra of *β*-CD-ENA (Fig. 3b) and *γ*-CD-ENA (Fig. 3c) mixtures confirming that aromatic ring is fully embedded into the CD cavity in both these cases. That the guest molecule enters the cavity from wider rim side, in both these cases, is ascertained by observation of cross peaks between H-3′ and benzylic protons (H-7) of enalapril. Since H-6′ which is positioned near the narrower rim of the CD also shows contacts with aromatic protons in the spectrum of *γ*-CD-ENA mixture, it can be said with great certainty that penetration of aromatic ring is quite deep in the case of *γ*-CD-ENA complex.

The most probable mode of penetration of aromatic ring into the α-CD cavity, which can explain the close proximity of H-2,4,6 of ENA with H-3′ of α-CD is shown as in Fig. 4 (Mode II). Though it is very clear that aromatic ring enters the cavity from wider side and is fully embedded in the CD cavity in the cases of *β*-CD-ENA and *γ*-CD-ENA complexes, it is not possible to predict, beyond doubt, the

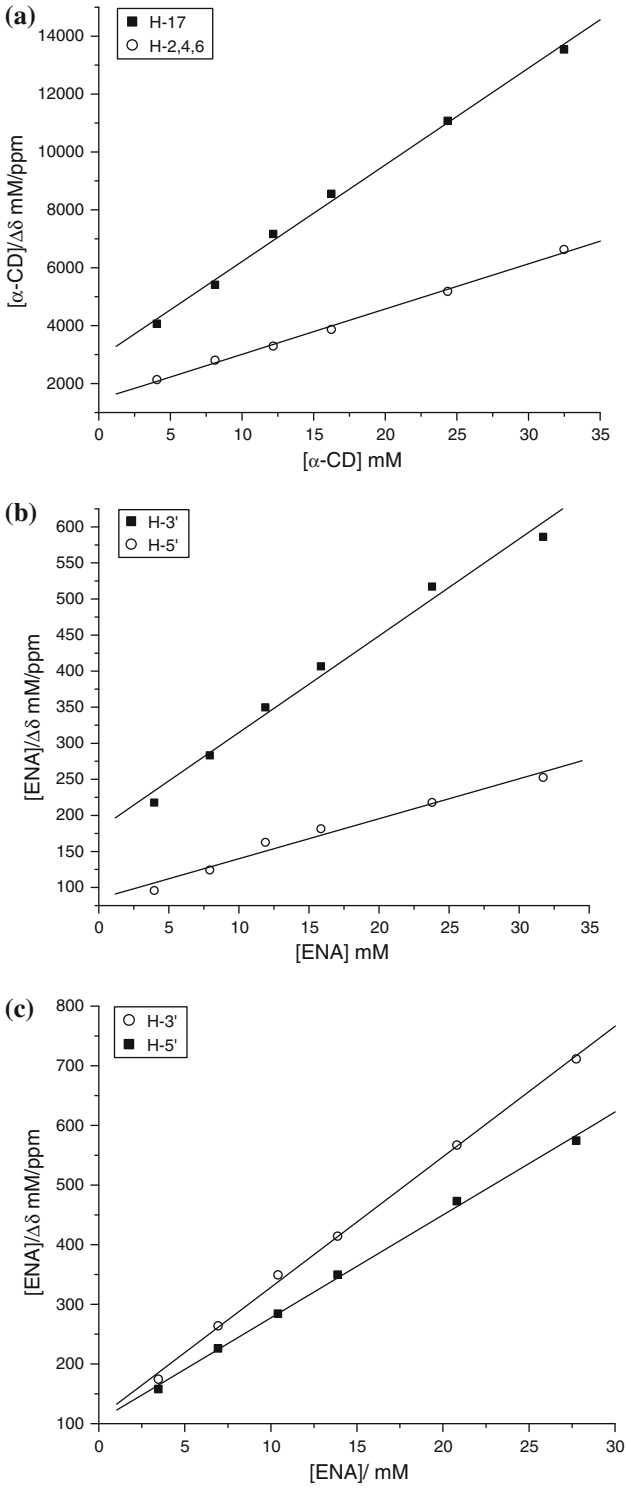


Fig. 2 Scott's plots for (a) H-17 (*filled square*) and H-2,4,6 (*open circle*) of ENA with increasing concentration of α-CD (b) H-3′ (*filled square*) and H-5′ (*open circle*) of *β*-CD with increasing concentration of ENA (c) H-3′ (*open circle*) and H-5′ (*filled square*) of *γ*-CD with increasing concentration of ENA

exact geometry of the complexes due to poor resolution of cross peaks. Two probable geometries (Modes I and II) can be considered for the complexes of ENA with *β*-CD and

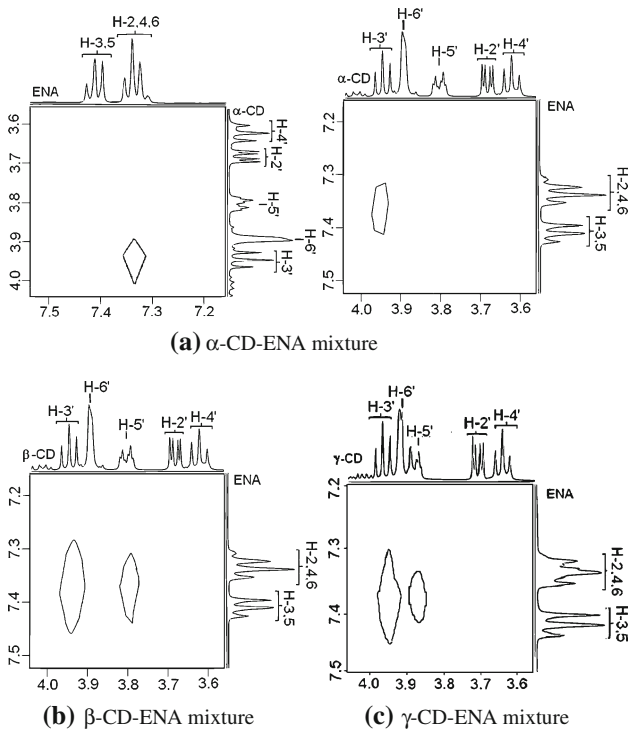


Fig. 3 Partial 2D ROESY spectra of mixtures of ENA with (**a**) α-CD (**b**) *β*-CD (**c**) *γ*-CD showing intermolecular interaction of aromatic protons of ENA with CD cavity protons

γ-CDs (Fig. 4). Of the two probable geometries, Mode I seems more favorable due to steric factors but this needs further confirmation. In order to fully establish the structures of these complexes, molecular modeling studies were performed.

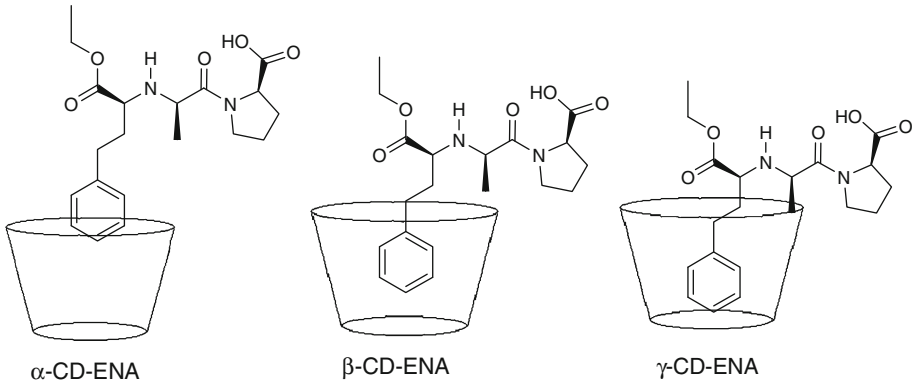
Computational studies of inclusion complexes of enalapril with cyclodextrins

Molecular mechanics studies of the complexation of enalapril with α-, *β*-, and *γ*-CDs using Allinger's MM2 force field [25] were carried out to fully establish the structures of inclusion complexes [26, 27]. All the calculations were performed without consideration of solvent because of the assumption that complexation of medium to large-sized organic hydrophobic substrates might lead essentially to non-hydrated inclusion compounds [28–30].

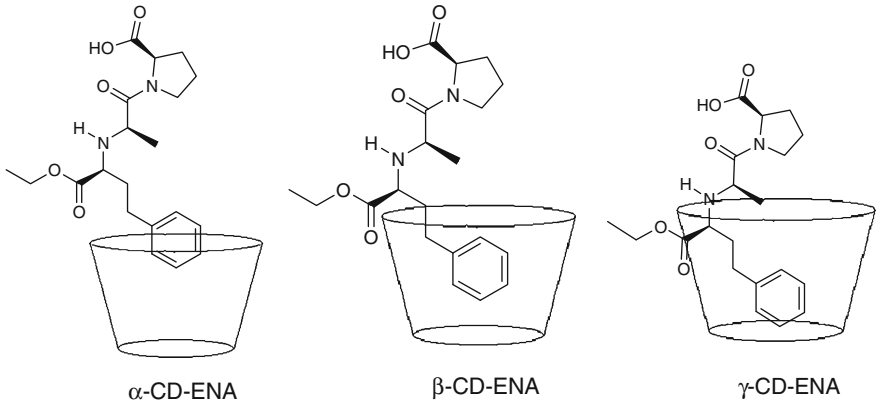
Calculations on the inclusion process

The geometries of CDs were obtained from their crystallographic structures deposited in the Cambridge databank. The published X-ray coordinates for uncomplexed hydrated CDs were used as starting point after removal of the water molecule coordinates. The structure of the enalapril was drawn using CS Chem3D Pro and its geometry was then minimized to a root mean square (RMS) value of

Fig. 4 Probable geometries of various ENA-CD complexes



Probable Structures of complexes by Mode I of entry of aromatic ring into the CD cavity



Probable Structures of complexes by Mode II of entry of aromatic ring into the CD cavity

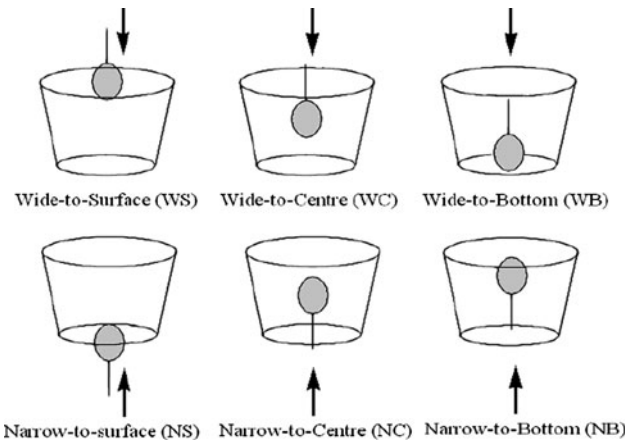


Fig. 5 Orientations of ligands used for molecular mechanics MM2 calculations

0.1 kcal/mol/Å with the MM2 force field under 300 K target temperature in vacuum. This geometry was always used in all calculations of inclusion complexes.

The MM2 calculations [26, 27] for the process of inclusion of enalapril into the CD cavity through both the entries have been carried out for all the possible binding sites of the enalapril. A total of six possible orientations for each binding site were studied (Fig. 5). The simulations were performed by manual insertion of the guest in the vertical position into the CD cavity in a perpendicular manner of its diameter. The total conformational energies of all the complexes are presented in Table 1.

MM2 calculations of α-CD-ENA complexes

The molecular mechanics (MM2) calculations of α-CD-ENA complexes showed that complexes in which ligand enters the cavity from the wider rim side were more stable, with a few exceptions, compared to those formed by the penetration of the ligand from narrower rim side. Moreover, complexes formed by penetration of the aromatic ring into the CD cavity were more stable than those formed by entry of the ethoxycarbonyl group or proline ring. The positioning of the aromatic ring (Mode I) near the wider rim and partially inside the cavity (WS) followed by MM2 calculations gave a complex having energy 107.0622 kcal/mol which reduced gradually when the ring was moved inside the cavity and the complex having total energy 91.2412 kcal/mol was obtained when the ring was positioned in the center of the cavity (WC). It was observed that minimization of the energy resulted in tilting of the aromatic ring (Fig. 6).

Table 1 Total conformational energies (kcal/mol) obtained with MM2 method for the complexes of enalapril with α-, β- and γ-CDs

CD	Ligand	WS	WC	WB	NS	NC	NB
α-CD	Ar (Mode I)	107.0622	91.2412	92.7145	95.5027	99.0553	101.0099
	Ar (Mode II)	88.6016	92.2714	81.5344[a]	105.7575	98.5241	683.2351
	Ethoxycarbonyl	97.7007	93.5294	93.2221	100.4018	99.7485	101.2480
	Proline	100.3501	105.4635	109.3470	98.4806	97.9620	123.1537
β-CD	Ar (Mode I)	112.6862	85.8396[a]	100.0263	107.0926	91.3152	92.3973
	Ar (Mode II)	97.4973	101.7070	659.4723	111.8197	113.4087	654.9920
	Ethoxycarbonyl	115.6140	108.9375	100.2895	109.9016	112.5257	109.1344
	Proline	118.3423	96.1943	90.7769	102.6312	99.3079	94.7300
γ-CD	Ar (Mode I)	149.7940	128.1628	116.9530[a]	132.7524	136.2366	124.5135
	Ar (Mode II)	135.6111	133.0817	131.7741	142.9356	131.0508	139.3523
	Ethoxycarbonyl	144.7725	132.5763	126.8047	143.1152	129.8977	130.3165
	Proline	141.7355	130.0426	123.1286	128.4737	125.2259	126.3302

[a] The most stable complex

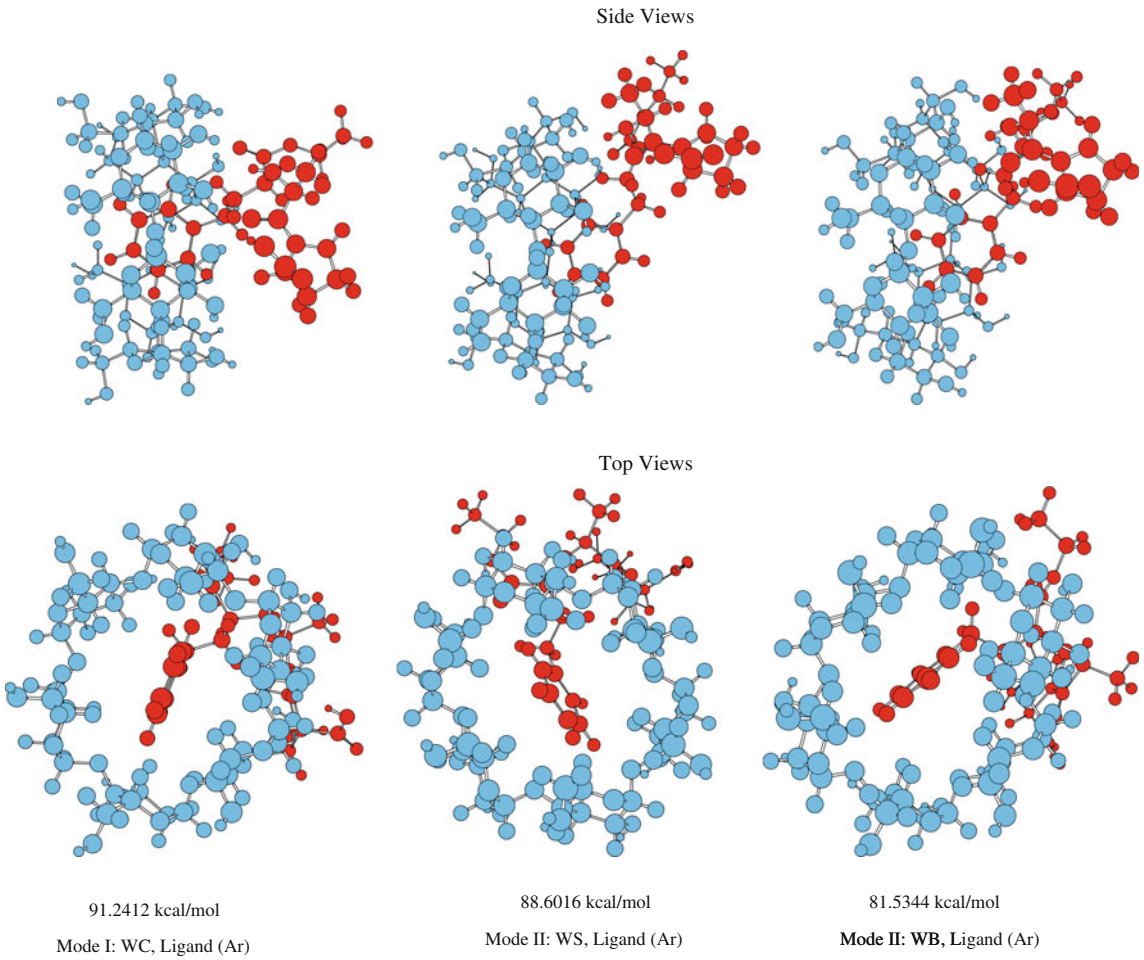


Fig. 6 Three most stable α-CD-ENA complexes

The complexes obtained by entry of the aromatic ring (Mode II) from the wider rim side were most favoured. The energy minimization of the complex formed by placing the aromatic ring near the mouth of wider cavity (WS) gave second most stable complex (total energy 88.6016 kcal/mol) in which the aromatic ring also had slightly tilted

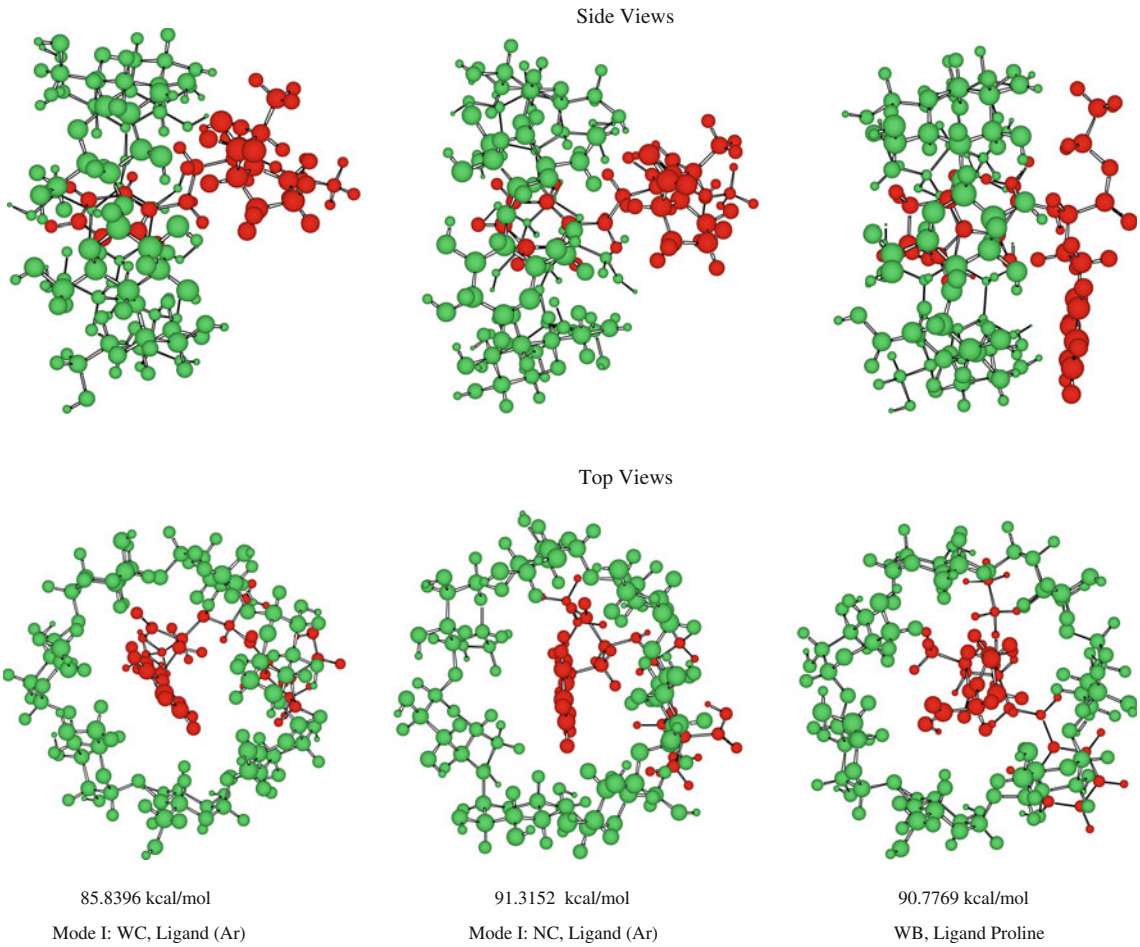


Fig. 7 Three most stable β-CD-ENA complexes

geometry (Fig. 6) which is not unexpected and has been reported [31]. It was observed that the aromatic ring was expelled from the cavity when it was placed from the wider rim in the center (WC) or near the bottom (WB) of the cavity. The most stable complex having a total energy of 81.5344 kcal/mol was obtained when MM2 calculations were made by placing the ring deep inside the cavity (WB) (Fig. 6). The results of MM2 confirm that aromatic ring enters the α-CD cavity as shown in Mode II (Fig. 4) which is also evident from 2D ROESY spectral data. The most probable structure of inclusion complex of enalapril with α-CD in aqueous solution is therefore the one in which aromatic ring is positioned near the wider rim in a slightly tilted position having total conformational energy of 81.5344 kcal/mol (Fig. 6).

MM2 calculations of β-CD-ENA complexes

The results of molecular mechanics MM2 studies of the β-CD-ENA complexes were quite different from those of α-CD-ENA complexes. Most stable complex (total energy 85.8396 kcal/mol) was obtained when aromatic ring was placed (Mode I) in the center of the CD cavity from wider rim side (WC). The positioning of the aromatic ring (Mode I) near the wider mouth of the cavity (WS) or deep inside the cavity (WB) gave quite unstable complexes having energies 112.6862 and 100.0263 kcal/mol, respectively. Similar pattern was observed when the aromatic ring (Mode I) entered from the narrower rim. The energies of these complexes were 107.0926 (NS), 91.3152 (NC) and 92.3973 kcal/mol (NB). The complexes formed by penetration of aromatic ring (Mode II) into the CD cavity from either side of the cavity were found to be less stable compared to those formed by penetration of the aromatic ring by Mode I.

The complexes obtained by entry of the ethoxycarbonyl group into the β-CD cavity from either side of the cavity were found to have high energies and are insignificant. However, complexes formed by penetration of the proline ring deep into the cavity were found to have relatively low energies, 90.7769 kcal/mol (WB) and 94.7300 (NB) kcal/mol. Three most stable β-CD-ENA complexes are shown in Fig. 7. These MM2 calculation results support the 2D ROESY results that the ring is positioned in the center of

the cavity and also that the aromatic ring enters from wider side of cavity. It can be concluded safely that the structure of β-CD-ENA inclusion complex is aqueous solution is the one having total energy 85.8396 kcal/mol (Fig. 7).

MM2 calculations of γ-CD-ENA complexes

Molecular mechanics MM2 calculations of the γ-CD-ENA complexes showed that most stable complexes are formed when the guest enters deep inside the cavity either from narrower or wider rim side. Moreover, complexes obtained when the guest entered from wider rim side were more stable than those resulting from penetration of guest from narrower side. The most stable complex was obtained when aromatic ring entered the cavity (Mode I) from wider rim side deep into the cavity (WB) and has total energy 116.9530 kcal/mol. Complexes involving aromatic ring (Mode II) were found to be least stable while those involving proline ring were found to be more stable than those involving ethoxycarbonyl group. Three most stable γ-CD-ENA complexes are depicted in Fig. 8. The most stable complex having total conformational energy 116.9530 kcal/mol (Fig. 8) probably represents the structure of ENA-γ-CD inclusion complex in aqueous medium.

Conclusion

It can be concluded that enalapril forms 1:1 inclusion complexes in aqueous medium with α-, β- and γ-CDs on the basis of chemical shift changes observed in the CDs cavity protons in the presence of enalapril maleate and NMR titration data. The structures of these inclusion complexes have been elucidated with the help of 2D ROESY spectral data which are well supported by molecular mechanics MM2 calculations. The complexation of ENA with α-CD results by partial entry of the aromatic ring into the cavity from the wider side by mode II (Fig. 4) in such a manner that only H-2,4,6 aromatic protons of ENA come in contact with H-3′ cavity proton of α-CD. The structures of complexes of ENA with β- and γ-CDs are also formed by penetration of aromatic ring into the CD cavity but the aromatic ring approaches the CD cavity by mode I (Fig. 4). The aromatic ring is positioned in the center of the β-CD

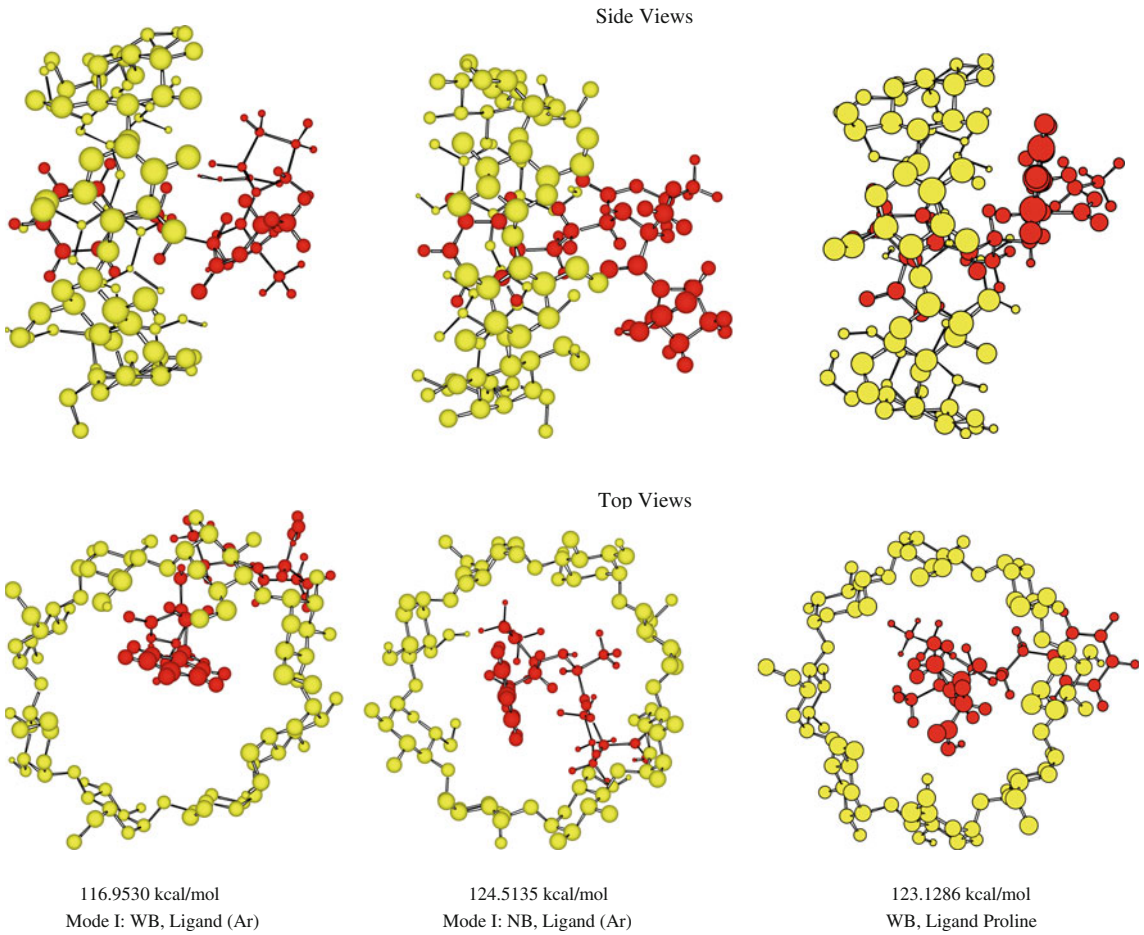


Fig. 8 Three most stable γ-CD-ENA complexes

cavity while it is deep inside the γ-CD cavity such that H-4 of ENA is slightly outside the bottom of cavity. The most probable structures of these inclusion complexes are shown in Fig. 6 (α-CD-ENA, total energy 81.5344 kcal/mol), Fig. 7 (β-CD-ENA, total energy 85.8396 kcal/mol) and Fig. 8 (γ-CD-ENA, total energy 116.9530 kcal/mol).

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