NMR spectroscopic study of inclusion complexes of cetirizine dihydrochloride and β -cyclodextrin in solution

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Abstract. Cetirizine dihydrochloride (CTZ), an antihistamine, forms two 1:1 inclusion complexes with β -cyclodextrin (β -CD) in aqueous solution as confirmed by detailed ^1H NMR, COSY and ROESY spectroscopic studies. The stoichiometry and overall binding constant of the complexes were determined by the treatment of ^1H NMR chemical shift change ($\Delta\delta$) data. Most of the CTZ protons exhibited splitting in the presence of β -CD.

Keywords: Cetirizine dihydrochloride, β -cyclodextrin, inclusion complex, NMR spectroscopy, ROESY, COSY

1. Introduction

Cetirizine dihydrochloride, (CTZ) is a second-generation, H₁-receptor antagonist, antihistamine drug with the amine piperazine in its molecule [1]. It is used to treat the symptoms of allergies, chronic urticaria, hay fever, angioedema, hives and as a treatment adjunct in asthma [2]. Chemically it is described as $(\pm)$ -[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride. It is a light sensitive drug and is used as a racemic mixture.

The study of inclusion complexes with cyclodextrins (CDs), especially β -CD, is a subject of great interest [3–5], because inclusion of the guest molecule, or part of it, included into β -CD cavity leads to altered physiochemical properties of the guest. The inclusion complexes of pharmaceutical compounds with CDs, therefore, have been extensively studied and utilized to improve the solubility [6], dissolution rate [7] and bioavailability of poorly water-soluble pharmaceuticals [8] and the stability of oxygen and light sensitive drugs [9].

CDs are toroidal or cone shaped molecules composed of six (α), seven (β) and eight (γ) D-glucopyranose units linked by α -(1, 4) linkages [3,10]. The H-3', H-5' and glucosidic oxygen are located inside the cavity which is relatively hydrophobic. The exterior of the CDs is fairly hydrophilic due to presence of large number of hydroxyl group. CDs, therefore, can accommodate a variety of non-polar molecules, or part of these, inside the cavity through non-covalent interactions, to form inclusion complexes [3,4,10].

There has been extensive work in the area of NMR analysis of CDs inclusion complexes and it is one of the most acceptable techniques for the study of inclusion complexes [11–13]. Evidence for the inclusion

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of the guest into the host CD cavity is obtained by simple ^1H NMR titration data. ^1H NMR spectra of mixtures of CD and guest molecule are recorded and changes in the chemical shift ($\Delta\delta$) for protons of both the host as well as guest are studied. When a guest is accommodated within the CD cavity, there is an upfield NMR shift in β -CD cavity protons while guest protons generally show downfield shift changes. These shift changes are attributed to the ring current effect of the aromatic guest. Information regarding the stoichiometry and association/dissociation constant of the complex can also be obtained by the treatment of simple ^1H NMR titration data.

2D ROESY [11–13] spectroscopy is a very useful technique for establishing the structures of CD inclusion complexes. NOEs cross correlation peaks between the guest and β -CD cavity protons (H-3', H-5') are observed in the ROESY spectrum, when the guest molecule is included into the β -CD cavity. According to the relative intensities of these cross peaks, it becomes possible to estimate the orientation of the guest molecule into the β -CD cavity. This information proves very helpful in determining the mode as well as depth of penetration of the guest and thus establishing the structure of the complex.

We are interested in the preparation and characterization of inclusion complexes of pharmaceutical compounds with β -CD in solution [14]. In continuation of our work, we report herein our results on the detailed study of the complexation between cetirizine dihydrochloride (CTZ) and β -CD in aqueous solution by NMR spectroscopic methods.

2. Results and discussion

All the ^1H NMR spectra were recorded on a JEOL α -500 MHz in D_2O at room temperature and their chemical shift values (δ) are reported in ppm. No external indicator was used and HDO signal at 4.800 ppm was used as internal reference throughout this work. ^1H NMR spectra of five samples of mixtures of CTZ and β -CD with CTZ/ β -CD molar ratios ranging from 0.2 to 1.2 were recorded. The overall concentration of β -CD was kept constant at 10 mM while that of CTZ was varied from 2.0 to 12.0 mM. Distinct peaks for bound and free form of the CTZ were not observed indicating that CTZ is undergoing rapid exchange between free and bind state on the NMR time scale.

The signals for β -CD protons, in spectra of mixtures, were identified with the help of 2D COSY spectrum. A cursory examination of the spectra of CTZ/ β -CD mixtures indicated highfield shift changes in the β -CD cavity protons while other β -CD protons showed relatively negligible shift changes.

The highfield shift of β -CD cavity protons, in the presence of CTZ, can be explained in terms of ring current effect of aromatic ring penetrating the β -CD cavity, thus confirming the formation of CTZ/ β -CD inclusion complex, in analogy to previous studies [15,16]. Expansions of the part of the spectra containing β -CD proton resonances, in the absence as well as in the presence of CTZ, are shown in Fig. 1 while their chemical shift change ($\Delta\delta$) data is given in Table 1.

The stoichiometry and the association constant (Ka) [17] of the CTZ/ β -CD complex/es were determined by Foster and Fyfe [18] method, an alternative solution of Benesi–Hildebrand equation [19]. In the Foster–Fyfe equation

$$\Delta\delta_{\text{obs}}/[G]_{\text{t}} = -Ka\Delta\delta_{\text{obs}} + Ka\Delta\delta_{\text{max}},$$

$\Delta\delta_{\text{obs}}$ is the observed chemical shift change for a given $[G]$, $[G]_{\text{t}}$ is the molar concentration of the guest, $\Delta\delta_{\text{max}}$ the chemical shift change between a pure sample of the complex and the free component at the saturation. A plot of $\Delta\delta_{\text{obs}}/[G]_{\text{t}}$ against $\Delta\delta_{\text{obs}}$ (referred as an x -reciprocal plot) should be linear for 1:1

Fig. 1. Expansion of part of ^1H NMR spectra (500 MHz) showing β -CD protons in the presence as well as in the absence of CTZ.

Table 1

^1H NMR chemical shift change ($\Delta\delta$, ppm) data for the β -CD protons in presence of CTZ

[CTZ]/[β -CD]	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
0.2	−0.071	0.004	−0.091	−0.008	−0.078	−0.005
0.4	−0.073	0.022	−0.164	−0.005	−0.139	0.003
0.6	−0.079	0.031	−0.222	−0.012	−0.188	−0.002
0.8	−0.086	0.030	−0.273	−0.018	−0.224	−0.008
1.2	−0.098	0.031	−0.353	−0.025	−0.282	−0.018

Negative values indicate upfield shift.

complex. The negative gradient is equal to Ka . This modification requires an extrapolation to infinitely dilute solution and the Ka is not dependent on the extrapolation. The overall association constant (Ka) was determined to be 70 M^{-1} which is the average of two Ks calculated from two plots (Fig. 2).

An unambiguous resonance assignment of CTZ protons was required to ascertain whether both or one aromatic rings are involved in complexation. The assignment of resonances of guest protons was made with the help of COSY and ROESY spectral data of CTZ, in the presence of β -CD because some of the signals which appeared completely merged in the spectrum of pure CTZ separated in the presence of β -CD helping in assignment.

The protons of the two aromatic rings appeared separately. The chlorophenyl ring protons were found resonating at a lower field compared to phenyl ring protons. The chlorophenyl ring protons were observed as a pair of partly overlapped doublets. The highfield ortho-coupled doublet at 7.604 was assigned to 1, 4-protons because they exhibited through space interaction with benzylic proton. The phenyl ring protons appeared as complex multiplet, totally integrating for five protons. In the presence of β -CD, the signals for both the aromatic ring protons exhibited splitting due to chiral recognition by β -CD. The expansions of aromatic region of spectra of CTZ in the presence as well as absence of β -CD are shown in Fig. 3.

2D ROESY spectrum was obtained for a mixture of β -CD and CTZ under spin lock conditions with a mixing time of 0.5 s. The spectrum (Fig. 4) exhibited strong cross correlation peaks between H-3' and H-5' of β -CD protons, located in the cavity, with the protons of both the aromatic rings of CTZ, confirming the penetration of both the phenyl as well as chlorophenyl rings into the β -CD cavity. More-

Fig. 2. Foster-Fyfe plot showing 1:1 stoichiometry for the β -CD/CTZ complexes.

Fig. 3. Expansions of part of ^1H NMR spectra (500 MHz) of CTZ protons in the presence as well as in the absence of β -CD.

over, strong cross peaks between H-4' of β -CD with the protons of both the aromatic rings were also observed. This is a clear indication that both the aromatic rings entered the β -CD cavity from wider rim side. Fig. 4 shows the part of the expansion of the 2D ROESY spectrum showing cross peaks between protons of aromatic rings and β -CD. The proposed structures for the two inclusion complexes formed between β -CD and CTZ are shown in Fig. 5.

In conclusion, ^1H NMR titration studies of mixture of cetirizine dihydrochloride (CTZ) and β -cyclodextrin (β -CD) in D_2O confirmed the presence of two 1:1 inclusion complexes formed by the

Fig. 4. Expansion of Part of 2D ROESY (500 MHz) spectrum (mixing time = 0.5 s) of mixture of β -CD and cetirizine dihydrochloride.

Fig. 5. Proposed structures of two 1:1 β -CD/CTZ inclusion complexes.

penetration of aromatic ring into the β -CD cavity. The stoichiometry and the overall binding constant (K_a) have been determined. The structures of the complexes have been proposed which are well supported by the ROESY spectral data.

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