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ORIGINAL ARTICLE

Complexation studies of pioglitazone hydrochloride and β-cyclodextrin: NMR (^{1}H, ROESY) spectroscopic study in solution

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Abstract Pioglitazone hydrochloride (PIO) is an agonist of the peroxisome proliferator-activated receptor γ (PPARγ), used to treat diabetes. ^{1}H-NMR spectroscopic analysis of varying ratios of β-cyclodextrin (β-CyD) and PIO in D_2O confirmed the formation of β-CyD–PIO inclusion complex in aqueous solution. The 1:1 stoichiometry of β-CyD–PIO inclusion complex was determined by Scott's plot method and binding constant (K_a) was calculated to be 155 M^{-1}. 2D ROESY experiments confirmed that the phenyl ring of PIO act as a guest and deeply penetrate in β-CyD cavity from wider as well as narrower rim side and form two 1:1 stable inclusion complexes. Some of the PIO protons exhibited splitting, in the presence of β-CyD, indicating chiral differentiation of PIO by β-CyD.

Keywords Pioglitazone hydrochloride · β-Cyclodextrin · NMR spectroscopy · ROESY · Inclusion complex

Introduction

Pioglitazone (PIO), chemically known as [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-]thiazolidinedione monohydrochloride, belongs to a different chemical class and has a different pharmacological action than the sulfonylureas, metformin, or the α-glucosidase inhibitors. PIO is an oral antibiotic drug effective for reactive hypoglycemia and aggravated glycemic metabolism associated with insulin resistance [1]. It is an agonist of the peroxisome proliferator-activated receptor γ (PPARγ) that raises HDL-cholesterol plasma in humans [2]. It is light sensitive, odourless, white crystalline powder and its solubility in water is 9.8 mg/mL.

Cyclodextrins (CyDs) are cyclic oligosaccharides composed of 6(α), 7(β) or 8(γ), α-(1→4)-linked glucose residues and characterized by a truncated cone shape [3]. Each of the chiral glucose units is in the rigid $^4C_1$-chair conformation, giving the macrocycle shape of a hollow truncated cone with all the secondary hydroxyl group, O(2)-H and O(3)-H (2n), located on the wider rim, while all the primary hydroxyl groups, O(6)-H (n), on the narrower rim [3]. The primary hydroxyls on the narrow side of the cavity can rotate, thus partially blocking the cavity, in contrast to the secondary hydroxyls, which are attached by relatively rigid chains and thus cannot rotate. The H-3′, H-5′ and glucosidic oxygen are located inside the cavity which is relatively hydrophobic. The other CyD protons (H-1′, 2′, 4′, 6′) are located outside the CyD cavity which is hydrophilic. As a consequence of these features CyDs can encapsulate a variety of hydrophobic molecule, or part of it, inside their cavity through non-covalent interactions to form inclusion complexes of host–guest type [3] (Fig. 1).

Complexation of pharmaceutical compounds with CyDs, to form a host–guest complex, results in altered physicochemical properties of the guest, like solubility, stability, volatility and masking of undesirable properties etc., which are desirable for their use as pharmaceuticals [3, 4]. Moreover, these host–guest complexes are considered as new entities and are required to be characterized for their approval as a drug. In pharmaceutical formulations, CyDs are generally used as solubilizers but also sometimes as stabilizers or to reduce local drug irritation [4].

There are various methods to study the inclusion complexation but ^{1}H-NMR spectroscopy is one of the most powerful analytical technique for investigating the

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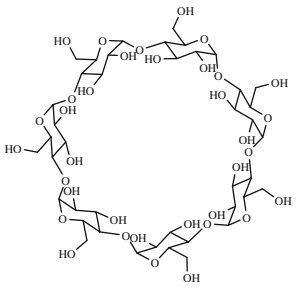
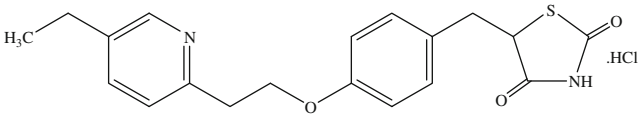


β-Cyclodextrin



Pioglitazone hydrochloride

Fig. 1 Structural representation of β-CyD (host) and PIO (guest) ligand

interaction between CyD and a guest [5, 6]. This technique provides a very clear picture of the inclusion complex formation by CyDs in solution phase and has also been used in chiral recognition and/or chiral discrimination studies [5, 6]. The stoichiometry and association/binding constant of the host–guest complex is readily achieved from NMR titration data [7].

The 2D ROESY has been found very helpful for the investigation of the interaction between CyD and guest molecule since the NOE cross peaks are observed between the protons that are closer than 0.4 nm in spaces in the ROESY spectrum [5, 6, 8]. It gives information about the part of the guest included inside the CyD cavity, the mode of penetration, i.e. either from narrower or wider rim side, the depth of penetration and orientation of the guest.

^{1}H-NMR spectra of mixture of CyD and guest molecule are recorded and changes in the chemical shift ($\Delta\delta$) of both host as well as guest are studied. The upfield shift of cavity protons namely H-3′ and H-5′ of CyDs and downfield shift of guest protons gives clear evidences for the formation of inclusion complex. Sometimes upfield shift changes of the guest protons are also observed.

The agonist of the piroxisome proliferator-activated receptor and efficient antidiabetic pioglitazone used as a guest molecule is undoubtedly interesting and important research object. In continuation of our work [9, 10], we report here the high-resolution solution structures of inclusion complex of pioglitazone hydrochloride and β-cyclodextrin by using various NMR techniques.

Materials and methods

The pioglitazone hydrochloride (PIO) was generously obtained from Amoli Organics, India, while the β-cyclodextrin (β-CyD) was obtained from Geertrui Haest, Cerestar Application Centre, Food & Pharma Specialities, France, and these were used as obtained. Four samples of mixtures of β-CyD and PIO were prepared with molar ratio [β-CyD]/[PIO] ranging from 0.2 to 3.7. The overall concentration of PIO was kept constant at 4.7×10^{-3} M while the concentration of β-CyD were varied. The chemical shift values are reported in δ (ppm) relative to the HDO peak at 4.800. All the ^{1}H-NMR experiments were recorded on Bruker AMX operating at 400 MHz spectrometer while the 2D ROESY spectrum was recorded on Bruker-500 MHz instrument in D_2O at room temperature. The mixing time for ROESY spectra was 500 ms under the spin lock condition. Chemical shifts changes ($\Delta\delta$) were calculated according to the formula: $\Delta\delta = \delta_{(\text{complex})} - \delta_{(\text{free})}$.

Stoichiometry of complex

The stoichiometry of the β-CyD–PIO complex was determined by using the well known Scott's plot method [11]. In Scott's equation,

$$[\text{CyD}]_t/\Delta\delta_{\text{obs}} = [\text{CyD}]_t/\Delta\delta_c + 1/K_a\Delta\delta_c$$

where, $[\text{CyD}]_t$ = molar concentration of the CyD; $\Delta\delta_{\text{obs}}$ = observed chemical shift difference for a given $[\text{CyD}]_t$ concentration; $\Delta\delta_c$ = chemical shift difference between pure sample of complex and the free component at the saturation.

The plot of chemical shift changes ($\Delta\delta$) for the PIO proton (H-1 and H-6, 9) against [β-CyD] in the form of [β-CyD]/$\Delta\delta$ versus [β-CyD] gave excellent linear fit (Fig. 2) confirming 1:1 stoichiometry for the β-CyD–PIO complex. The overall binding constant $K_a = 155\ \text{M}^{-1}$, and it is estimated by the way that the slope of the plot of [β-CyD] is thus equal to $1/\Delta\delta_c$ and intercept with the vertical axis to $1/K_a\Delta\delta_c$.

Results and discussion

The assignment of the β-CyD protons was made with the help of their characteristic shapes, position of signals and ^{1}H-NMR spectral data. Expansions of part of the spectra showing β-CyD protons, in the presence as well as in the absence of PIO, are shown in Fig. 3. A detail inspection of ^{1}H-NMR spectra of mixtures of β-CyD and PIO, indicates

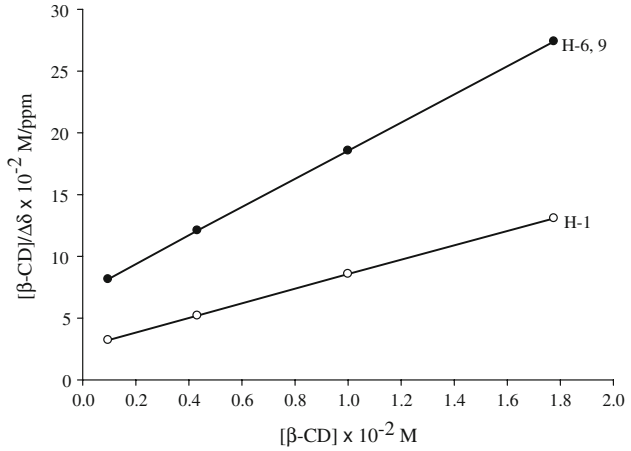


Fig. 2 Scott's plot for the β-CyD–PIO complex, confirming 1:1 stoichiometry

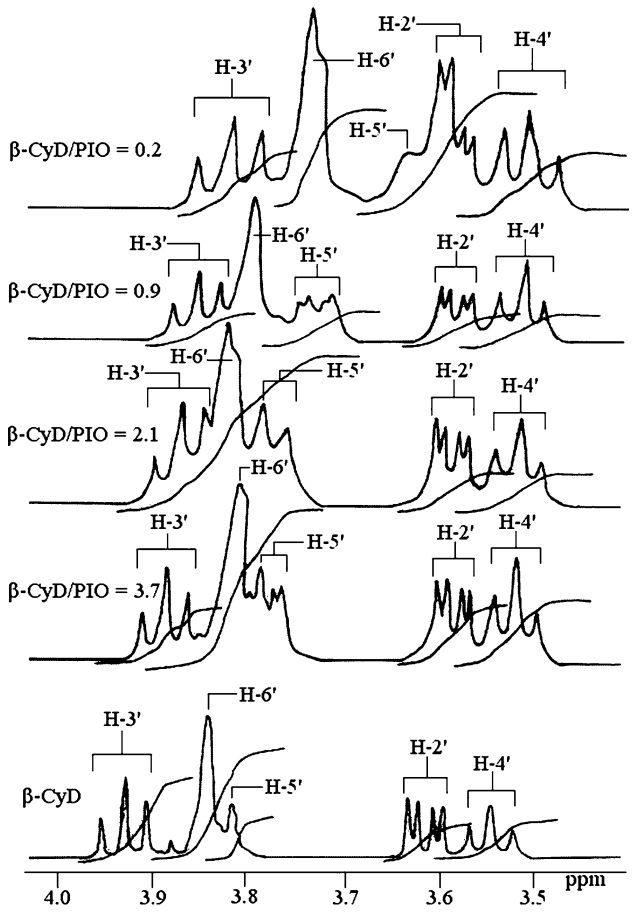


Fig. 3 Expended part of the ^{1}H-NMR spectra (400 MHz) of β-CyD protons in the presence, as well as in the absence, of PIO

that the β-CyD cavity protons (namely H-3′ and H-5′) influences significant upfield shift changes. Other β-CyD protons (namely H-1′, 2′, 4′, 6′) shows insignificant shift changes. Moreover, the chemical shift changes for H-5′ were more pronounced compared to H-3′. The most influenced β-CyD cavity protons (H-3′ and H-5′) which show highfield shift changes and concomitant shift changes in the signals for PIO protons in the β-CyD–PIO mixtures can only be attributed to the ring current effect of aromatic ring penetrating the β-CyD cavity, thus, confirming the formation of β-CyD–PIO inclusion complex in analogy to previous studies [5, 6, 9, 10].

To know the clear structure of the β-CyD–PIO complex, an ambiguous resonance assignment of PIO protons is required. The assignment of resonances of PIO protons was made with the help of ^{1}H-NMR spectra performed for a β-CyD–PIO mixtures. Part of the ^{1}H-NMR spectra showing aromatic regions of PIO, in the presence as well as in the absence of β-CyD is given in Fig. 4.

As expected, the signals for the pyridine ring protons appeared downfield compared to phenyl ring. All the protons of the substituted pyridine ring are more deshielded than the other ring and therefore well separated. One singlet at $\delta = 8.431$ was assigned for H-1 proton of pyridine ring. A doublet of doublet at $\delta = 8.325$ was assigned for H-3 signal, due to the strong coupling with H-2 (d, $\delta = 7.843$) and weak coupling with H-1. Two doublets at $\delta = 6.837$ and 7.156 belong to H-6, 9 and H-7, 8 assigned for phenyl ring, totally integrating for four protons, respectively. The chemical shift change data for the aromatic protons of PIO protons in the presence of β-CyD is given in Table 1.

In the presence of β-CyD, all of the aromatic protons of PIO significantly shifted downfield and exhibited splitting, indicating some chiral differentiation of PIO by β-CyD [12]. Other PIO protons shows insignificant shift changes. These observations clearly indicates the involvement of aromatic ring/s in complexation but which of these aromatic ring/s penetrates into β-CyD cavity cannot be

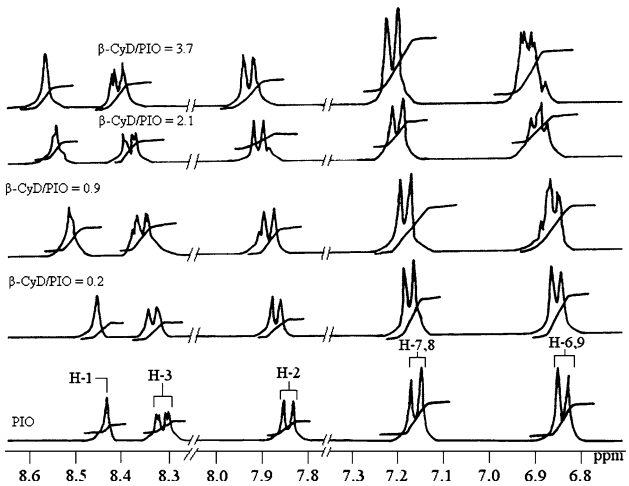


Fig. 4 Expanded part of the ^{1}H-NMR spectra (400 MHz) of aromatic protons of PIO in presence, as well in the absence, of β-CyD

Table 1 ^{1}H-NMR (400 MHz) chemical shift change ($\Delta\delta$) data of the aromatic protons of PIO in presence of varying amount of β-CyD

β-CyD/PIO	H-1	H-3	H-2	H-7, 8	H-6, 9
0.2	0.0321	0.0162	0.0188	0.0125	0.0125
0.9	0.0688	0.0350	0.0375	0.0223	0.0271
2.1	0.1138	0.0625	0.0688	0.0375	0.0522
3.7	0.1375	0.0961	0.1069	0.0563	0.0659

confirmed by this data, because mostly, all the protons of the guest, and not only the part that enters the β-CyD cavity, show downfield shift changes upon complexation with CyDs [13].

To ascertain whether phenyl or pyridine ring was involved in complexation, 2D ROESY spectrum (τ_m = 500 ms) was performed on β-CyD–PIO mixture. The ROESY spectrum of β-CyD–PIO mixture exhibited intermolecular cross peaks between phenyl ring protons (H-6, 9 and H-7, 8) and β-CyD cavity protons (H-3′ and H-5′). Other PIO ring protons did not show any cross peaks with β-CyD cavity protons. Expansions of the part of the ROESY spectrum of β-CyD–PIO mixture, showing cross correlation peak between aromatic protons of PIO and β-CyD cavity protons is given in Fig. 5.

On the basis of 1:1 stoichiometry, ^{1}H-NMR titration results and 2D ROESY spectroscopic data, it can be argued that only phenyl ring penetrates in the β-CyD cavity resulting in the formation of 1:1 β-CyD–PIO complex. As both the cavity protons of β-CyD (H-3′ and H-5′) show the cross peaks with H-6, 9 and H-7, 8, the penetration of phenyl ring is suggested to be from wider (H-3′ side) as well as narrower rim (H-5′ side) of β-CyD. Thus only two 1:1 β-CyD–PIO inclusion complexes with similar geometry were considered. The involvement of other PIO ring in β-CyD–PIO complexation was ruled out on the basis of 2D ROESY spectral data. The structure of the two 1:1 β-CyD–PIO inclusion complexes was therefore, characterized as shown in Fig. 6.

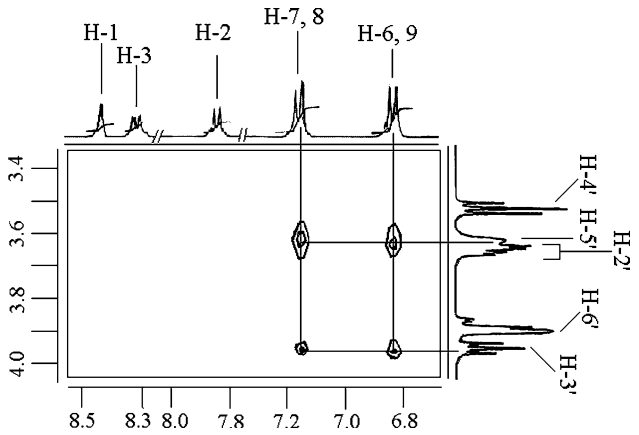


Fig. 5 Expanded region of 2D ROESY spectrum (500 MHz) of β-CyD–PIO mixture showing cross peaks of aromatic ring protons with β-CyD cavity protons

Conclusion

The ^{1}H-NMR analysis of pioglitazone hydrochloride (PIO) in the presence of β-cyclodextrin (β-CyD) in D_2O at room temperature confirmed the formation of 1:1 β-CyD–PIO inclusion complex, in which only phenyl ring acts as guest and deeply penetrates in the β-CyD cavity. The stoichiometry and association constant were determined using Scott's method by the treatment of ^{1}H-NMR titration data. Subsequently, 2D ROESY spectral data was analysed to proposed the structure of two possible 1:1 β-CyD–PIO inclusion complexes. The splitting of some of the signals of PIO in the presence of β-CyD suggests some chiral differentiation of PIO by β-CyD.

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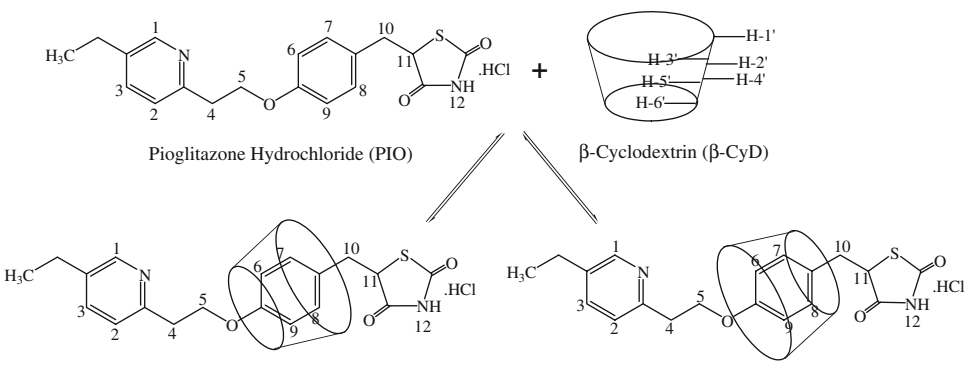

Fig. 6 Possible structures of two β-CyD–PIO inclusion complexes

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