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Inclusion Complexes of Cyproterone Acetate with Cyclodextrins in Aqueous Solution

SANDRINE HENRY DE HASSONVILLE[1]*, BRUNO PERLY[2], GÉRALDINE PIEL[1], THIERRY VAN HEES[1], VALÉRY BARILLARO[1], PASCAL BERTHOLET[1], LUC DELATTRE[1] and BRIGITTE EVRARD[1]

[1]*Department of Pharmaceutical Technology, University of Liège, Belgium;* [2]*Service de Chimie Moléculaire, CEA Saclay, F-91191 Gif-sur-Yvette Cedex, France*

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Abstract

Cyproterone acetate (CPA) is a steroidal antiandrogen with a progestogenic activity. Given that this molecule has a very poor water solubility (2.1 μg/mL), different cyclodextrins (CDs) were tested to form inclusion complexes and to increase solubility. Two different techniques were compared to study the affinity between CPA and CDs: phase-solubility studies and NMR spectroscopy. The stoichiometry and the stability constant could be determined for most complexes with the aid of phase-solubility studies. The greatest increase in solubility was achieved with the methylated β-CDs, but hydroxypropylated β- and γ-CDs also gave enhanced solubilities. ^{1}H-NMR studies showed a solubility increase similar to that found with phase-solubility studies. The proof of inclusion in the 2,6-dimethyl-β-CD (DIMEB) was shown by ^{1}H-NMR and t-ROESY spectra.

Introduction

CDs are well known for increasing water solubility of hydrophobic compounds by inclusion into their cavity. Various steroids have shown a good affinity for both β- and γ-CDs and their synthetic derivatives [1, 2]. CPA is a particular steroid that has an antiandrogenic and a progestogenic activity (Figure 1). It is used by women for the treatment of hirsutism, severe acne, androgenetic alopecia, and by men, for the treatment of hypersexuality or prostate cancer. CPA is very poorly water-soluble (2.1 μg/mL). To increase its solubility, we investigated the ability of CPA to form inclusion complexes with several derivatives of β- and γ-CDs.

Phase-solubility is one of the most frequently used methods to study the affinity between drugs and CDs [3]. In this work, phase-solubility studies will be compared with NMR spectroscopy to study the CPA-CDs inclusion complexes in solution. The aim of this research is to find which CD is the most efficient for increasing the solubility of CPA, to demonstrate CPA inclusion into the CD cavity as well as to determine the stoichiometry and the stability constant of the complexes.

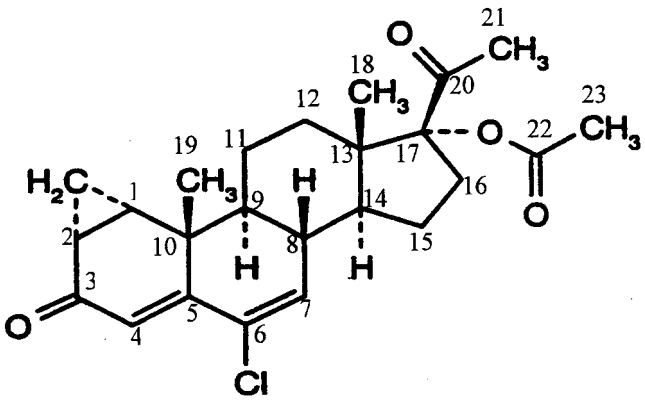

Figure 1. Chemical structure of CPA.

Experimental

Materials

CPA was obtained from Sicor (Mexico). β-CD was obtained from CNI (France). Hydroxypropyl-β-CD (HP-β-CD) was a gift from Roquette (France); sulfobutylether-7-β-CD (SBE$_7$-β-CD) was kindly supplied by Cydex (Kansas); randomly methylated β-CD (RAMEB), γ-CD and hydroxypropyl-γ-CD (HP-γ-CD) were generously given by Wacker Chemie GmbH. 2,6-dimethyl-β-CD (DIMEB), trimethyl-β-CD (TRIMEB), monoamine-β-CD

* Author for correspondence: Service de Technologie Pharmaceutique, ULG, CHU, Tour 4, 4000 Liège, Belgium. E-mail : shassonville@ulg.ac.be

and monoamine-DIMEB were synthesized at CEA Saclay (France).

Phase solubility studies

Solubility studies were performed as described by Higuchi and Connors [3]. Excess amounts of CPA were added to increasing concentrations (0–200 mM) of CDs in aqueous solutions. The CDs tested were: β-CD, HP-β-CD, SBE$_7$-β-CD, RAMEB, γ-CD and HP-γ-CD. After shaking at 25 °C for 24 h, the undissolved CPA was removed by filtration through a 0.45-μm filter (Millipore PVDF) and the solutions were assayed for CPA content by HPLC.

NMR studies

CDs solutions were prepared in D_2O at a 10-mM concentration. As water-solubility of CPA is too low, spectra of CPA alone could not be performed in D_2O. For assignment of the protons, NMR spectra of CPA were performed in DMSO. Solutions containing CPA and CDs were prepared as in experiments for phase-solubility studies. Excess amounts of CPA were added to a 10-mM CD solution and after shaking at 25 °C for 24 h, the suspensions were filtered (Millipore PVDF 0.45 μm). The different CDs tested were: α-CD, β-CD, HP-β-CD, RAMEB, DIMEB, TRIMEB, monoamine-β-CD, monoamine-DIMEB, and γ-CD.

All NMR experiments were performed on a Bruker DRX500 spectrometer operating at 500 MHz for proton. The temperature was set to 298K. Calibration was achieved using the residual resonance of the solvent as secondary reference (4.80 ppm for HDO) corresponding to external TMS at 0 ppm. For T-ROESY experiments, a 300-msec mixing time was used. All processings were done on Silicon Graphics INDY data stations using the WINNMR program from Bruker.

Results and discussion

Phase-solubility studies

Figure 2 shows the diagrams obtained with the different CDs. β-CD shows the lowest solubility enhancement, probably because it is poorly soluble by itself. Solubility increases much more using its synthetic derivatives (HP-β-CD, SBE$_7$-γ-CD and RAMEB). The most dramatic increase is obtained with RAMEB (Table 1). The solubility of CPA in a 200-mM RAMEB solution (9 mg/mL) is more than 4000 times higher than its intrinsic solubility (2.1 μg/mL). Following the classification of Higuchi and Connors, β-CD and HP-β-CD showed A_L diagrams (Table 2). They thus give complexes of stoichiometry 1:1. The SBE$_7$-β-CD diagram is classified as A_N type and the stoichiometry cannot be established. For the RAMEB, there is an A_P diagram and the stoichiometry will be determined by the method of Higuchi and Connors. The diagram of the γ-CD is particular because it begins like an A_P type, but from a concentration of 100 mM, the complex precipitates. Therefore the diagram

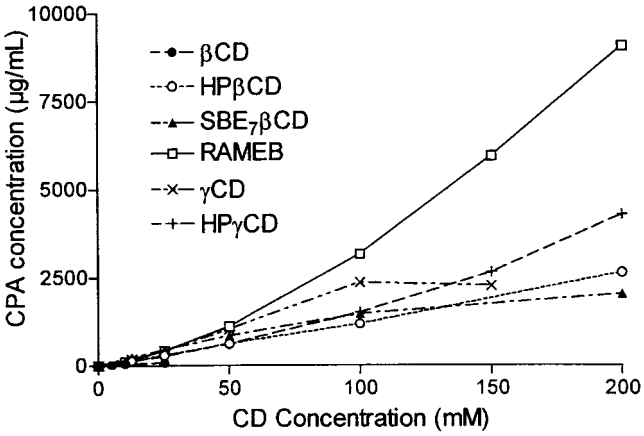

Figure 2. Phase-solubility diagrams of CPA with various cyclodextrins.

Table 1. Maximum solubility increase of CPA obtained with different cyclodextrins

CD	[CD] tested	Max. solubility	Solubility increase
β-CD	0–15 mM	86 μg/mL	41
HP-β-CD	0–200 mM	2648 μg/mL	1243
SBE-β-CD	0–200 mM	2034 μg/mL	954
γ-CD	0–150 mM	2369 μg/mL	1112
HP-γ-CD	0–200 mM	4291 μg/mL	2015
RAMEB	0–200 mM	9071 μg/mL	4258

is classified as B_S type. HP-γ-CD increases the solubility a bit more than the HP-β-CD, but less than the RAMEB (Table 1). The HP-γ-CD diagram is, like for the RAMEB, classified as an A_P diagram.

For A_L diagrams (β-CD, HP-β-CD), stability constants (K_c) were calculated from the following equation:

$$K_c = \frac{S_t - S_0}{S_0 \cdot ([\mathrm{CD}]_t - (S_t - S_0))}$$

where S_t is the solubility of the active substance (free +complexed); S_0, intrinsic solubility of the active substance; $[\mathrm{CD}]_t$, CD concentration (free +complexed).

In order to determine the stoichiometry of the complexes with RAMEB, γ-CD and HP-γ-CD, $\frac{S_t - S_0}{[\mathrm{CD}]_t - (S_t - S_0)}$ was plotted in function of $([\mathrm{CD}]_t - (S_t - S_0))$ (Figure 3) and the stability constant was calculated from the following equation:

Table 2. Stoichiometry and stability constant of CPA-CD complexes in aqueous solution, determined by the phase-solubility studies

CD	Diagram	Stoichiometry	K1:1	K1:2
β-CD	A_L	1:1	2685 M^{-1}	–
HP-β-CD	A_L	1:1	6374 M^{-1}	–
SBE-β-CD	A_N	?	–	–
γ-CD	B_S	1:1, 1:2	6619 M^{-1}	12 M^{-1}
HP-γ-CD	A_P	1:1, 1:2	4555 M^{-1}	7 M^{-1}
RAMEB	A_P	1:1, 1:2	5929 M^{-1}	19 M^{-1}

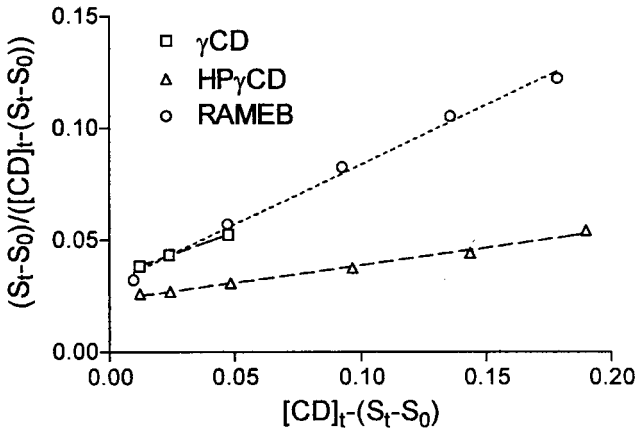

Figure 3. Representation of $([CD]_t - (S_t - S_0))$ in function of $\frac{S_t - S_0}{[CD]_t - (S_t - S_0)}$ to determine the stoichiometry and the affinity constant of CPA complexes with γ-CD, HP-γ-CD and RAMEB.

$$\frac{S_t - S_0}{[CD]_t - (S_t - S_0)}$$

$$= K_{1:1} \cdot S_0 + K_{1:1} \cdot K_{1:2} \cdot S_0 \cdot ([CD]_t - (S_t - S_0))$$

For the three CDs, straight lines were obtained, which means that CDs form complexes of stoichiometry 1:1 and 1:2. Stability constants (K1:1 and K1:2) were then calculated and their values are given in Table 2.

For RAMEB, γ-CD and HP-γ-CD, K1:1 is about 4000–7000 M^{-1} and K1:2 is very low (less than 20 M^{-1}). The inclusion compound is thus mostly composed of a 1:1 stoichiometry complex. The complex 1:1 with HP-β-CD has a K1:1 value close to that of other CDs, and K1:1 of β-CD is a bit lower.

NMR studies

Depending on the CD, the solubility of CPA was different and signals were more or less noticeable on the spectra. For α-CD and TRIMEB, no CPA signals could be detected. Thus these CDs do not increase CPA solubility. For the other CDs, the solubility increase could be calculated by the ratio between the digital integration of relevant lines from the CPA and from the CD (e.g., H-4 or H-7 signals of CPA and H-1 signals of CD). Results are given in Table 3. HP-β-CD, RAMEB and γ-CD seem to enhance solubility in the same way, i.e., about 0.3 mM. The most dramatic solubility enhancement of CPA is obtained with DIMEB. The solubility of CPA was found to be approximately 1 mM, which is more than three times higher than that obtained with RAMEB. However, with the monoamine derivatives, solubility enhancement is less significant than that observed with the corresponding CDs.

As expected, the cavity of α-CD is too small to form inclusion complexes with CPA. Nevertheless, both β-CD and γ-CD present a cavity with a suitable size. The substitution of these CDs with hydroxypropylated or methylated derivatives allows increasing the affinity for CPA. The behaviour of methylated derivatives is particularly interesting. Actually, solubility enhancement is the best for DIMEB, substituted in position 2 and 6, is intermediate for RAMEB, which is randomly substituted in 2, 3 and 6, and is negligible for TRIMEB, which is substituted in 2, 3 and 6. The free hydroxyl group in position 3 thus seems to play a key role in the formation of a soluble inclusion complex.

Table 3. Quantity of CPA dissolved (mM) in a 10-mM CD solution calculated from the integrations of signals in ^{1}H-NMR or assayed by HPLC

	^{1}H-NMR	HPLC
α-CD	0	–
β-CD	0.13	0.14
γ-CD	0.35	0.33
HP-β-CD	0.26	0.25
DIMEB	1.00	–
RAMEB	0.32	0.31
TRIMEB	0	–
β-CD monoNH$_3^+$	0.06	–
DIMEB monoNH$_3^+$	0.55	–

With monoamine derivative, the presence of an amine function gives a more hydrophilic character to the cavity, which decreases the affinity for the CD.

As DIMEB gave the best results, further investigation of the CPA-DIMEB complex was undertaken. The spectrum of DIMEB alone was compared with that of DIMEB in presence of CPA (Figure 4). The signals corresponding to H-3 and H-5 protons are weakly shifted upfield. As shifts were small, the proof of inclusion could not be provided. Given these results, T-ROESY spectra were performed to provide more information on inclusion. Figure 5 shows T-ROESY spectrum of the complex CPA-DIMEB and interactions between H-3 and H-5 protons of CD and CPA protons. The correlation spots indicated interactions between H-4 proton of CPA and H-3 and H-5 of the CD, between H-7 proton of CPA and H-3 CD proton (small circle). Other interactions between H-3 and H-5 of CD were also observed with a large number of CPA protons (large circle), especially with CH_3 in position 18, 19, 21, 23, H-1, H-2 and one of the H of the CH_2 in 1,2α. These results demonstrate the inclusion of CPA in the CD cavity. As different parts of the molecule can fit into the CD cavity, further investigations must be undertaken to determine the most probable configuration for the CPA-DIMEB complex.

Comparison of the two techniques

Table 3 shows a comparison between the quantity of CPA dissolved in a 10-mM CD solution calculated from phase-solubility studies or from ^{1}H-NMR spectra. The values obtained for the four CDs compared are relatively similar. Both methods are thus able to measure the solubility enhancement of compounds in a CD solution. As HPLC is more sensitive than NMR, phase-solubility studies, followed by HPLC assay, is interesting for poorly soluble drugs. Actu-

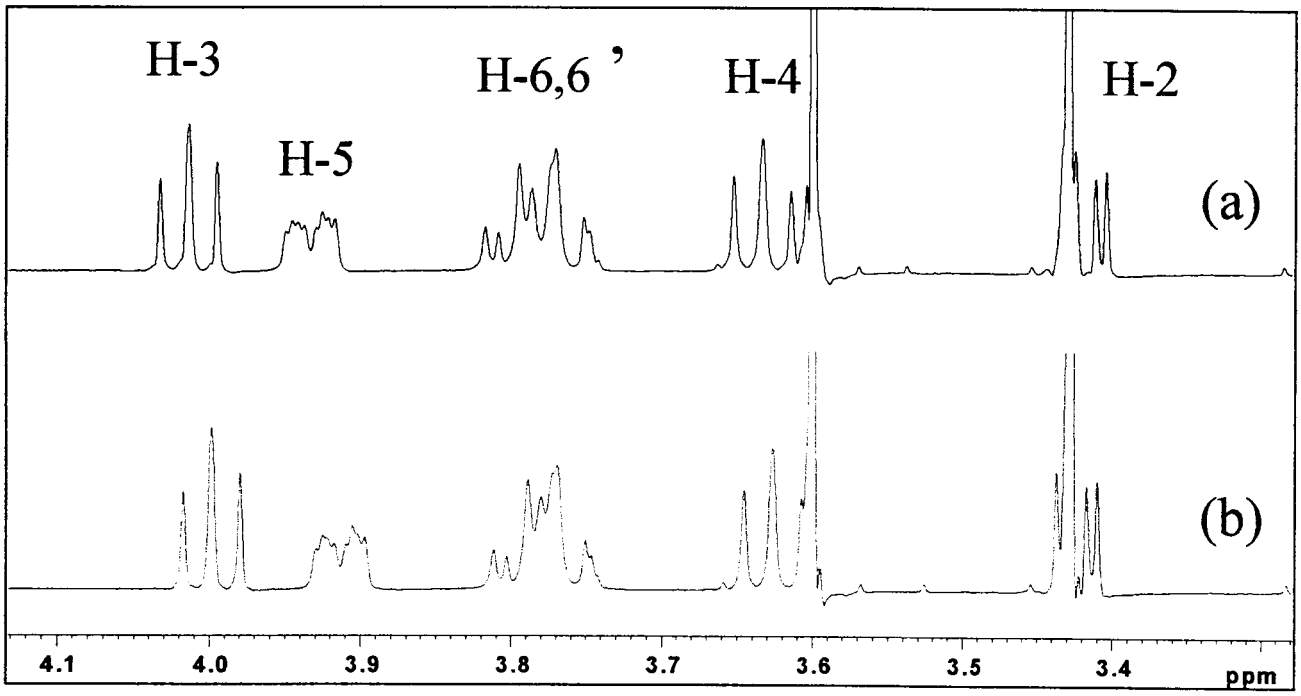

Figure 4. Partial ^{1}H-NMR spectra of 10 mM solutions (a) DIMEB and (b) DIMEB in the presence of CPA dissolved in D_2O with assignment of the signals of the CD moiety.

ally, until now it has not been possible to perform the spectra of poorly soluble drugs in D_2O by ^{1}H-NMR. Nevertheless, ^{1}H-NMR is a better method to demonstrate the inclusion of the complex and to determine its structure.

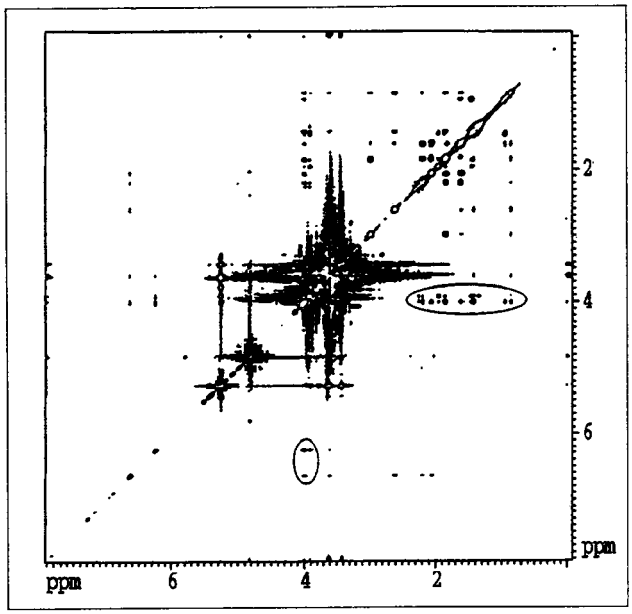

Figure 5. Contour plot of T-ROSEY spectrum of CPA dissolved in a 10-mM DIMEB solution in D_2O (interactions between CPA and CD protons are circled).

Conclusion

Phase-solubility diagrams and ^{1}H-NMR spectroscopy are two complementary techniques to study the inclusion complexes of CPA in CDs. The best solubility enhancements were achieved with DIMEB, RAMEB, HP-γ-CD and HP-β-CD. The stability constants of these complexes were approximately 4000–7000 M^{-1}. This indicates that CPA has a moderate affinity for the CDs. The study of the CPA-DIMEB complex with ^{1}H-NMR and T-ROESY spectra demonstrates that CPA is partially included into the CD cavity. Further analyses must be undertaken to more intensively study the structure of the complex.

Acknowledgements

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