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ORIGINAL ARTICLE

Inclusion complex characterization between progesterone and hydroxypropyl-β-cyclodextrin in aqueous solution by NMR study

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Abstract The aim of this work is the determination of the molecular association of progesterone (P) with hydroxypropyl-β-cyclodextrin (HPβCD) in aqueous solution. The stoichiometry and the binding constants of the inclusion complex were calculated using NMR techniques.

Keywords Inclusion complex · NMR · Cyclodextrin · HPβCD · Progesterone

Introduction

Progesterone (P) is a natural hormone utilized as a drug to control the reproductive function and for postmenopausal therapy. With the current administration routes (oral, cream, intramuscular oily solution or water suspension injections) P has low bioavailability. In addition, the oral route has a limited usefulness because of its short half-life of P and the extensive degradation after absorption. Therefore the injection route should be the preferred choice and this encourages research of new injectable pharmaceutical forms with decreased patient complications.

The use of cyclodextrin to increase the water solubility of P was described by Pitha [1]. To improve solubility, stability and bioavailability of drugs, and to avoid the toxic effects due to the parenteral application, hydroxypropyl-β-cyclodextrin (HPβCD) represents a valuable alternative to βCD. The evidence of the inclusion complex formation between progesterone and β-cyclodextrin/β-cyclodextrin derivatives is described using different physic–chemical methods [2, 3].

In this work, NMR techniques were used to characterize hydroxypropyl-β-cyclodextrin/progesterone complexes. The formation and stoichiometry of the inclusion complexes were determined using the *continuous variation method*, through monitoring of proton shifts of P during NMR titration. Moreover, the hydroxypropyl-β-cyclodextrin/progesterone complex has been also investigated by solid state ^{13}C NMR spectroscopy technique.

Materials and methods

Progesterone (P) was an industrial batch from Diosynth (The Netherland), hydroxypropyl-β-cyclodextrin (HPβCD) was supplied by Roquette. The P/HPβCD powder for injection was prepared according to the European patent deposit n.05108494.5 [4]. All NMR solution experiments was performed at 11.4 T on a Bruker Avance500 spectrometer equipped with a TXI 5 mm probe at 313 K. A proton NMR spectrum of the Progesterone sample was recorded from a saturated D_2O solution. The HPβCD and P/HPβCD samples were characterized by homo and heteronuclear experiments (Bruker library: COSY, TOCSY, HSQC and HMBC) with a 7.0 mM D2O solution. External trimethylsilyl propionate sodium salt was used as chemical shift reference.

A deuterium oxide solution containing a constant concentration of P (8.9×10^{-5} mmol/ml) was titrated

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with increasing amounts of HPβCD (from 0 to 5.48×10^{-4} M). Due to the complexity of HPβCD spectrum, in P/HPβCD complex only the ^{1}H shifts of P were measured to study the inclusion process.

Solid state ^{13}C Cross Polarization Magic Angle Spinning with dipolar decoupling (^{13}C CP-MAS) spectra were recorded with a Bruker ASX300 spectrometer operating at 75.47 MHz equipped with 4 mm CP-MAS probe. The spin rate was 8000 Hz.

Results and discussion

Progesterone (Fig. 1) was characterized by monodimensional ^{1}H spectrum as its low solubility in water did not allow to collect 2D data. The ^{1}H NMR spectrum of P was recorded from a saturated D_2O solution. The corresponding ^{1}H chemical shifts are reported in Table 1 [5].

Since no characterization of HPβCD/P complex was reported in the literature, we performed a NMR study of the complex. Proton and carbon P signals were assigned using homonuclear (COSY and TOCSY) and heteronuclear (HSQC and HMBC) experiments. All proton and carbon chemical shifts are shown in Table 1. Moreover, NMR spectroscopy gives information on the relationship between host and guest molecules by observing the shift of both progesterone and HPβCD signals.

Proton shift of signals between P and the complex are observed. Particularly, shifts of H4, H18, H19 and H21 of P in the complex with HPβCD suggest that both side of the steroid molecule are included in the cyclodextrin. The latter observation was supported by the integration ratio between H4 of P and the anomeric signals of HPβCD, indicating a 2:1 HPβCD/P complex.

The comparison of the HSQC spectra of HPβCD and of the 2:1 complex (Fig. 2) displays a significant shift of both H3 and H5 signals of the cyclodextrin,

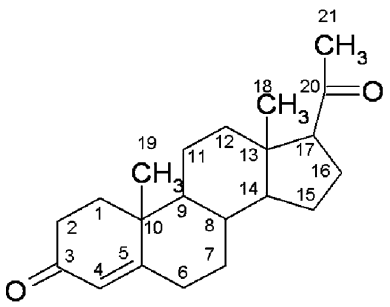

Fig. 1 Progesterone structure

Table 1 ^{1}H and ^{13}C chemical shifts (ppm) of P in a D_2O solution and in the 2:1 HPβCD/P complex

Atom	P (ppm)		P in 2:1 complex	
	^{1}H	^{13}C (ppm)	^{1}H (ppm)	
1	2.17 (β) 1.75 (α)	38.52	2.10–1.98 (β)	1.78–1.61(α)
2	2.50 (β) 2.32 (α)	36.14	2.60–2.46 (β)	2.36–2.33(α)
4	5.90	125.05		
5		180.04		
6	2.58 (β) 2.45 (α)	35.66	2.36–2.33 (β)	2.36–2.33(α)
7	2.04 (β) 1.14 (α)	34.80	1.93–1.87 (β)	1.11–1.02(α)
8	1.77	38.26		
9	1.05	56.95		1.11–1.02(α)
10		41.68		
11	1.80 (β) 1.63 (α)	24.05	1.78–1.61 (β)	1.78–1.61(α)
12	2.16 (β) 1.60 (α)	41.41	2.10–1.98 (β)	1.78–1.61(α)
13		47.20		
14	1.29	58.79		
15	1.88 (β) 1.39 (α)	26.84	1.25 (β)	1.78–1.61(α)
16	2.13 (β) 1.82 (α)	26.09	2.75 (β)	2.60–2.46(α)
17	2.61	66.36		
18	0.77	16.64		
19	1.32	20.33		
21	2.16	33.98		

therefore confirming formation of an inclusion complex.

In order to calculate the kinetic of the inclusion process, a NMR titration was performed. The guest (P) concentration was kept constant (8.9×10^{-5} mmol/ml) and 12 NMR spectra were recorded, allowing the chemical shifts to be monitored as a function of increasing concentrations of the HPβCD (Fig. 3).

The ^{1}H NMR complexation induced shifts (CIS) of the most intense signals are reported in Table 2.

The complexation induced proton shifts observed by increasing the amount of HPβCD are reported in Fig. 4. The complexation shifts were simulated using different approaches. Firstly, the presence of only a 1:1 complex was considered. Then, we moved to a two step equilibrium characterized by two apparent formation constants, K_{11} and K_{21}. All proton complexation shifts of P were utilized in the non-linear least-square fitting procedure [6].

A multiple equilibrium involving 1:1 and 2:1 guest/host complex was found; with K_{11} value of about 8000 M^{-1} and , K_{21} about 100 M^{-1}.

Moreover, to confirm the inclusion of P into HPβCD, the ^{13}C CP-MAS solid state spectra of P, HPβCD and their complex sample were recorded. The spectrum of P shows very sharp and well defined signals, a peculiarity for a crystalline and rigid structure (Fig. 5).

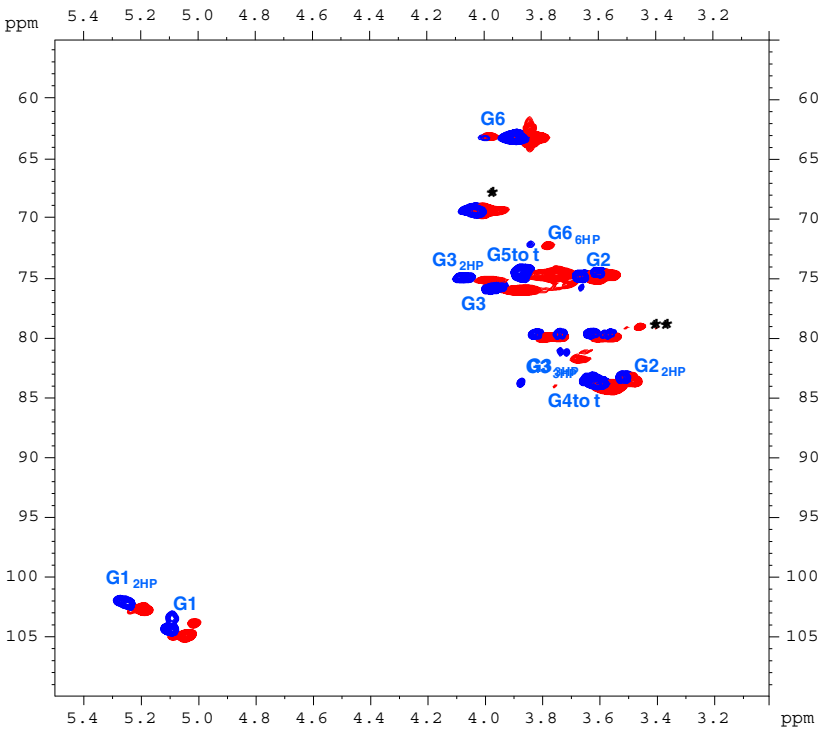

Fig. 2 Overlap of HSQC spectrum of HPβCD (blue) and its complex with P (red). (* CHOH Hydroxypropyl groups;** CH2OH Hydroxypropyl groups)

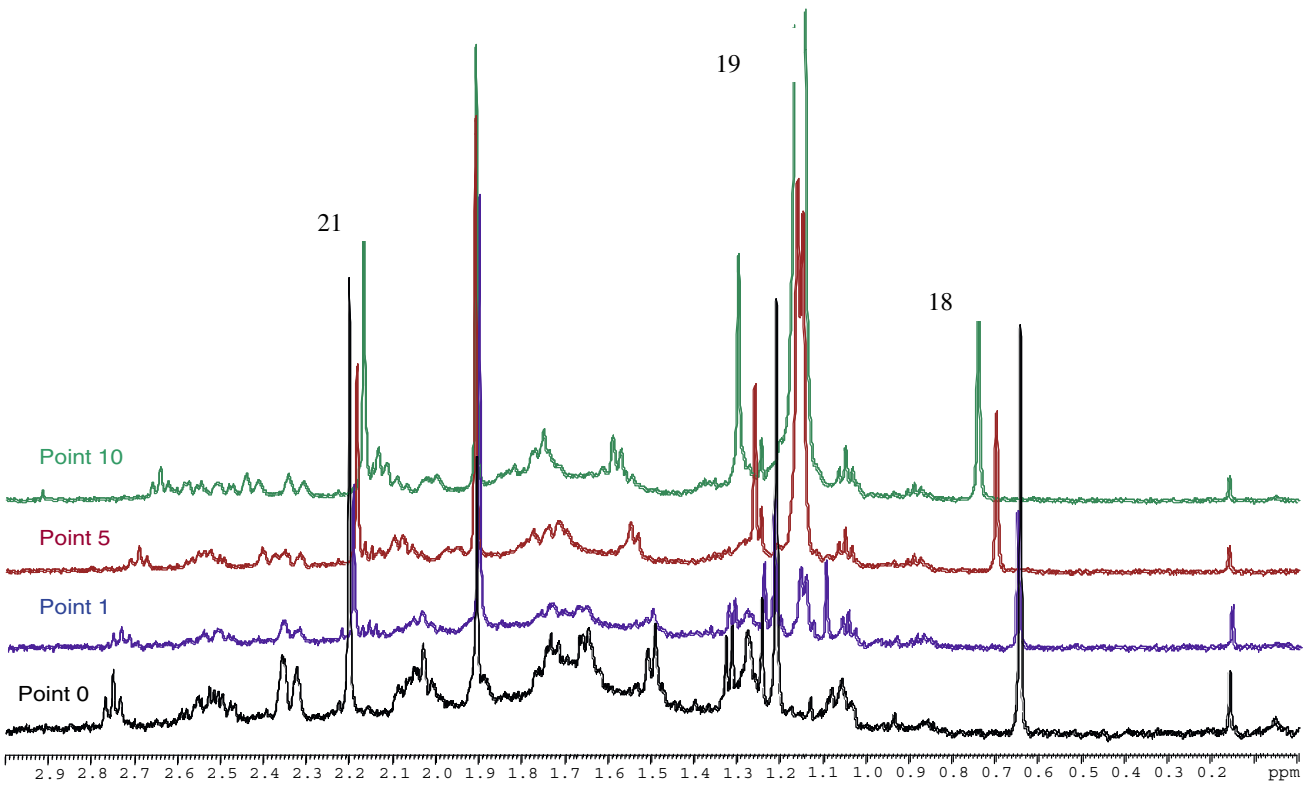

Fig. 3 Expansion of the 3-0 ppm region for the spectra recorded for points 0, 1, 5, and 10 of the titration.

Table 2 P chemical shifts in 2:1 complex (ΔHz)

HPβCD (M)	Peak 19	Peak 18	Peak 21	Peak 4
0.000	0.000	0.000	0.000	0.000
8.55×10^{-6}	4.170	4.900	2.615	3.255
1.71×10^{-5}	6.920	8.020	3.715	5.270
3.42×10^{-5}	14.620	16.270	5.915	11.505
5.10×10^{-5}	17.185	19.935	7.745	13.885
6.84×10^{-5}	24.390	27.320	8.980	19.805
1.03×10^{-4}	26.120	29.055	9.995	20.805
1.37×10^{-4}	32.405	36.440	13.250	26.355
1.71×10^{-4}	36.070	40.105	14.530	29.290
2.14×10^{-4}	38.920	43.685	15.900	31.585
2.56×10^{-4}	43.680	48.260	16.825	35.245
3.42×10^{-4}	44.275	49.040	17.695	35.845
5.48×10^{-4}	50.055	55.375	20.350	40.340

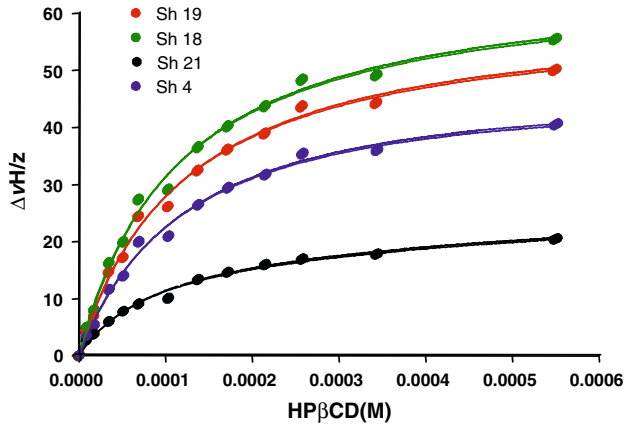

Fig. 4 complexation shift of protons induced by HPβCD addition; - non-linear square fitting

By contrast, the solid state spectrum of HPβCD (Fig. 6) consists of broad signals indicating an amorphous structure with very high mobility.

The CP-MAS spectrum of the 2:1 complex shows the signals related to HPβCD and aliphatic carbons of P (Fig. 7).

The progesterone peaks are broad, reflecting loss of order structure. Moreover, the peaks attributed to carbonyl groups (peak 3 and 20), and to the double bond (peak 4 and 5) are not present in the spectrum recorded with the same instrumental parameters used for P. This means that, due to higher rigidity of the included aromatic region more than for the rest of the P structure, the relaxation time of these atoms in the 2:1 complex increases. This confirms that both sides of P are included in the cavity of two HPβCD molecules.

Conclusions

In this work the HPβCD/P complex was characterized by NMR spectroscopy with the attribution of the most significant ^{1}H and ^{13}C signals. The complexation-induced shift of H3 and H5 protons of HPβCD, observed in the HSQC experiment, are those expected as a result of interaction with those protons, which are oriented towards the cyclodextrin cavity, thus, confirming a host/guest interaction. Such a conclusion is also in agreement with spectra with CP MAS spectrum of the solid complex, where signals associated with the crystal structure of P are no longer observable. Solution spectra confirm that two opposite sides of a single

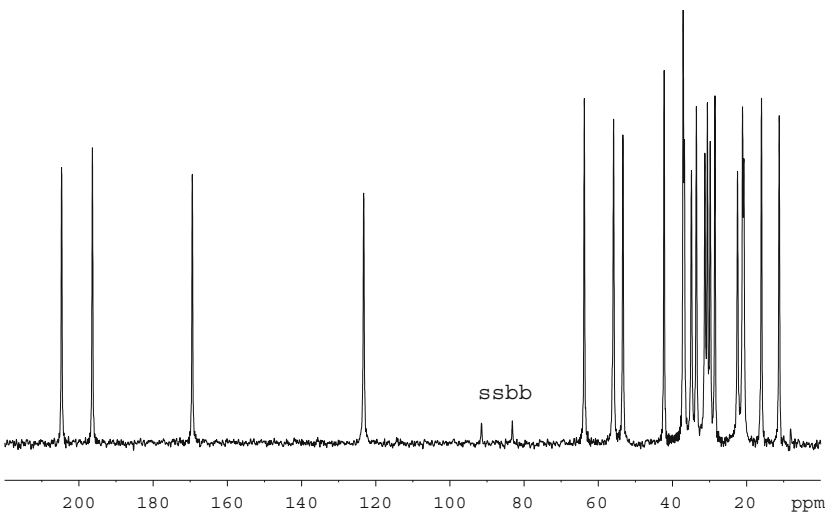

Fig. 5 Progesterone ^{13}C CP-MAS spectrum (ssbb: spinning side band)

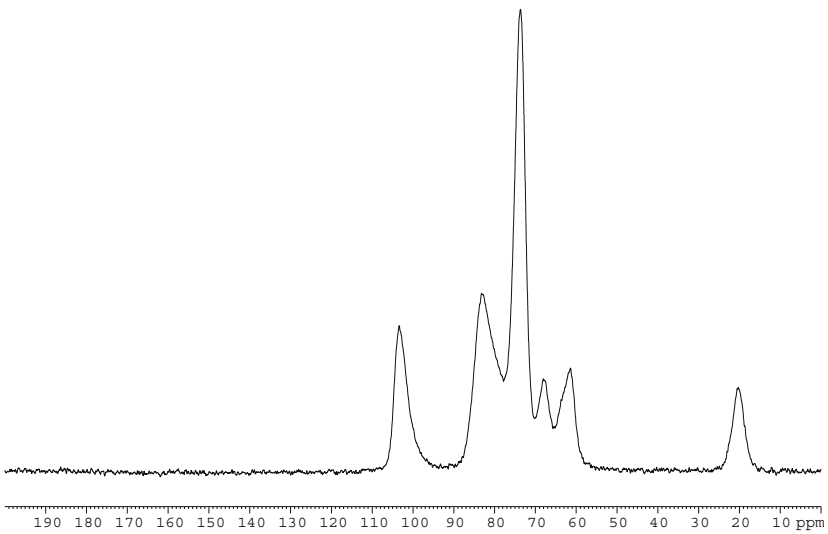

Fig. 6 HPβCD ^{13}C CP-MAS spectrum

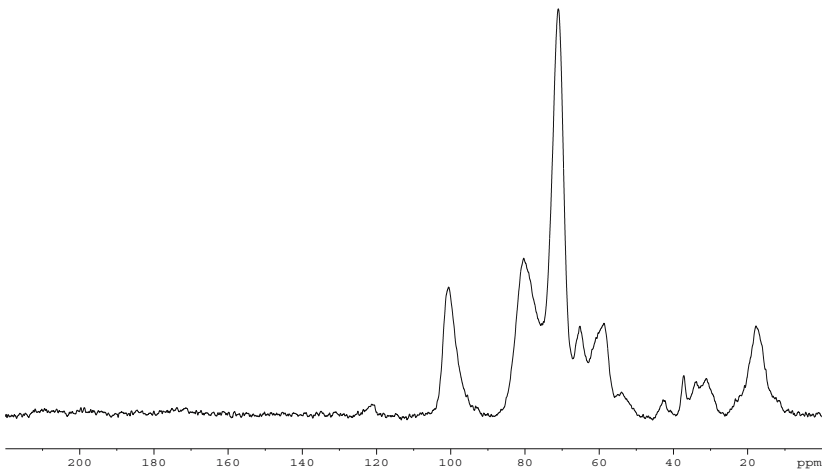

Fig. 7 2:1 complex ^{13}C CP-MAS spectrum

P molecule are included in two HPβCD. The complexation induced proton shifts of P, measured upon addition of increasing amounts of HPβCD, agree with the hypothesis of a multiple equilibrium involving both 1:1 and 2:1 species.

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