One- and two-dimensional NMR study of complexation of ursodeoxycholic acid with β-cyclodextrin

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The interaction of ursodeoxycholic acid (UDCA) with β-cyclodextrin (β-CD) is investigated through one- and two-dimensional (ROESY) NMR spectroscopy. The relative orientation of host and guest is unequivocally established: the aliphatic side chain of UDCA enters the torus of β-CD on the side of the secondary hydroxy groups.

Introduction

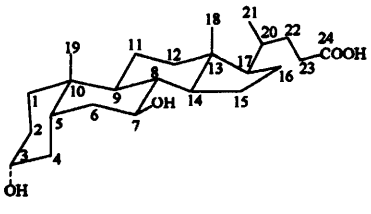
Cyclodextrins are cyclic glucose polymers in which glucopyranose units are bonded through α-(1,4) linkages. The torus-like shape allows them to include a variety of guests[1] and this property is used to enhance the bioavailability of poorly soluble drugs.[2]

Some bile acids are successfully employed in the medical field (therapy of liver disease,[3] cholesterol gallstone dissolution[4]) and their solubility in water improves in the presence of β-cyclodextrin (cycloheptaamylose)[5] and 2-hydroxypropyl-β-cyclodextrin.[6] The geometry of the inclusion complexes of bile acids and β-cyclodextrins has been hypothesized on the basis of the shifts of both ^1H[5] and of ^{13}C[6] NMR signals. Both approaches suggest the introduction of the aliphatic chain into the inner cavity of cyclodextrin, but the relative orientation proposed for the host and the guest molecules is opposite in the two cases.

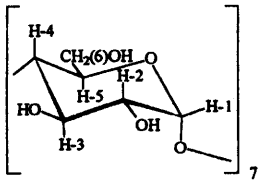
In the present paper a one- and two-dimensional NMR study on the interaction between ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid, UDCA) and β-cyclodextrin (β-CD) is reported. This work clearly shows that the aliphatic chain of UDCA enters the β-CD cavity from the secondary hydroxy rim, in contrast to previous findings.[5]

Results and discussion

It is known that, passing from the free to the complexed state, the ^1H and ^{13}C NMR chemical shifts of host and guest molecules change and from these changes information about the geometry of inclusion complexes can be drawn.[7-9] This classical approach can be applied only to β-CD, due to the very poor solubility of UDCA in D_2O, the solvent employed for this study. The changes in β-CD ^1H and ^{13}C NMR chemical shifts ($\Delta\delta$) upon complexation are reported in Tables 1 and 2, respectively. Table 1 shows that the two inner protons of β-CD undergo a negative shift, higher for 5-H than for 3-H. The same trend, observed in closely related complexes, led Tan and Lindenbaum[5] to infer that the bile acids enter the β-CD cavity from the primary hydroxy rim side. Table 2 shows that ^{13}C NMR chemical shifts are more sensitive to complexation than ^1H ones and that C-1, C-6 and C-4 are more influenced than C-3 and C-5 by UDCA. Moreover, $\Delta\delta$ C-3 is higher than $\Delta\delta$ C-5 and this behaviour is opposite to that of the corresponding protons. Therefore, it is difficult, at this stage to draw geometrical information about the inclusion complex only on the basis of ^1H and ^{13}C NMR data. To our knowledge, these $\Delta\delta$ ^{13}C values are the highest reported in the literature for β-CD, but their relative trend is very similar to that observed for the terfenadine–β-CD inclusion complex.[9]



UDCA



β-CD

Alternatively, the nuclear Overhauser effect[10] is a powerful tool for detecting the proximity of two protons and it is often applied to complex systems in its two-dimensional version (NOESY). When this experiment (250 ms mixing time) was performed on the complex UDCA–β-CD, the absence of cross-peaks revealed $\omega_0\tau_c$ *ca.* 1.1. A rotating-frame Overhauser effect spectroscopy (ROESY) study[10,11] was therefore undertaken. A standard pulse sequence was utilized and various experiments, with different settings of the spin-lock field frequency, were performed in order to distinguish between genuine ROE and false homonuclear Hartmann–Hahn (HOHAHA)-transferred ROE[10,12] (both positive with respect to the negative diagonal) cross-peaks. This HOHAHA-transfer within the glucose unit is particularly effective when the spin-lock field frequency is in the region of 4.8–2.4 ppm. UDCA proton assignments, known in methanol solution,[13] were checked through total correlation spectroscopy (TOCSY)[14] and ^1H, ^{13}C multiple-quantum inverse-detection experiments, HMQC[15] and HMBC,[16] directly on the complex in D_2O. The 7:1 ratio of β-CD signals with respect to those of UDCA makes the long-range connectivities difficult to detect in the HMBC experiments. Almost only those relative to the three methyls and the relayed carbons were clearly detected.

Several genuine intermolecular ROE cross-peaks appear when the spin-lock field is set at 0.8 ppm (Fig. 1): between 21-CH_3 (1.01 ppm) of UDCA and 5-H (and, to a lesser extent, 6-H) of β-CD, between 18-CH_3 (0.88 ppm) of UDCA and 3-H of β-CD but not between 19-CH_3 (1.05 ppm) of UDCA and any proton of β-CD. Other significant ROE cross-peaks are found between 23-H (2.32 and 2.16 ppm) of UDCA and 6-H and 5-H

Table 1 ^{1}H NMR chemical shifts (δ, ppm) of β-cyclodextrin (β-CD) in the absence and in the presence of ursodeoxycholic acid (UCDA)

	1-H	2-H	3-H	4-H	5-H	6-H	6'-H
$\delta_{\beta\text{-CD}}$ [a]	5.10	3.68	4.00	3.62	3.90	3.92	3.90
$\delta_{\beta\text{-CD-UDCA}}$ [a]	5.12	3.71	3.97	3.68	3.83	3.92	3.91
$\Delta\delta$ [b]	+0.02	+0.03	−0.03	+0.06	−0.07	0	+0.01

[a] 1.6×10^{-2} M solutions in D_2O. [b] $\Delta\delta = \delta_{complexed} - \delta_{free}$.

Table 2 ^{13}C NMR chemical shifts (δ, ppm) of β-cyclodextrin (β-CD) in the absence and in the presence of ursodeoxycholic acid (UDCA)

	C-1	C-2	C-3	C-4	C-5	C-6
$\delta_{\beta\text{-CD}}$ [a]	104.67	74.91	75.92	83.96	74.66	63.13
$\delta_{\beta\text{-CD UDCA}}$ [a]	105.25	74.93	76.21	84.29	74.80	62.72
$\Delta\delta$ [b]	+0.58	+0.02	+0.29	+0.33	+0.14	−0.41

[a] 1.6×10^{-2} M solutions in D_2O. [b] $\Delta\delta = \delta_{complexed} - \delta_{free}$.

Table 3 Summary of the intermolecular ROE cross peaks between β-cyclodextrin (β-CD) and ursodeoxycholic acid (UDCA) protons [a]

	UDCA protons							
β-CD protons	12β-H	15β-H/16α-H	17-H	18-CH_3	20-H	21-CH_3	22s-H	23s-H
3-H	++	++		+++				
5-H	+	+		+	++	+++	++	+[b]
6s-H			+		+	++	+	+

[a] See also text and Fig. 1. The relative strength of cross peaks is indicated by '+', '++' and '+++'. [b] These weak cross peaks are not visible in the contour plot reported in Fig. 1. Nevertheless, they can be evidenced with lower levels.

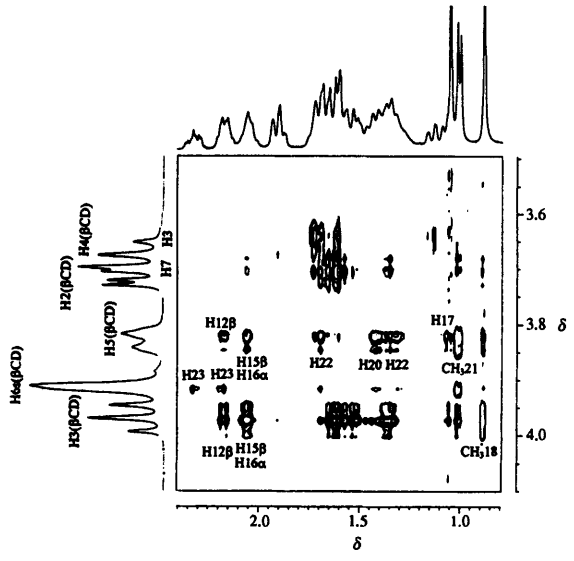


Fig. 1 Partial ROESY NMR spectrum of the supramolecular complex β-CD–UDCA (intermolecular ROE region)

of β-CD, and between 12β-H (2.17 ppm), 15β-H and/or 16α-H (both at 2.06 ppm) of UDCA and 3-H (also 5-H, to a lesser extent) of β-CD. Cross-peaks between 22-H (1.70 and 1.30 ppm), 20-H (1.42 ppm) and 17-H (1.07 ppm, hidden under 1β-H 1.12 ppm and 19-CH_3) of UDCA and 5-H of β-CD are more intense than those between the same protons and 6-H of β-CD. The ROESY region corresponding to 3-H of β-CD and UDCA's protons between 1.3 and 1.8 ppm is too complex to be unequivocally assigned. The cross-peaks in the region 3.6–3.75 ppm/1.8–1.3 ppm are due to intramolecular ROEs between 3-H (3.63 ppm) and 7-H (3.70 ppm) and other UDCA protons. Intermolecular ROE cross peaks are summarized in Table 3.

In conclusion, the ROESY experiment provides us with detailed information on the proximity of host and guest: protons 12-H, 18-CH_3, 16-H and/or 15-H of UDCA are closer to 3-H, whereas 21-CH_3, 17-H, 20-H, 22-H and 23-H are closer to 5-H and 6-H of β-CD. These findings unambiguously show that UDCA enters the inner cavity of β-CD on the side of the secondary hydroxy groups with its aliphatic chain. The interaction of UDCA with β-CD involves, besides the chain, the two rings close to it, at least up to 12-H. These observations parallel and confirm those derived from previous studies in CD_3OD[6] on the complexation of 2-hydroxypropyl-β-cyclodextrin with UDCA and other bile acids, based on the analysis of the changes of ^{13}C NMR chemical shifts of the guests upon complexation. On the basis of the ROESY results, the $\Delta\delta$ of ^{13}C NMR signals of β-CD can be interpreted as follows. The strong shifts of C-1 and C-4, are probably due to conformational changes affecting the exocyclic C-1–O–C-4 bonds upon complexation, whereas the shielding experienced by C-6 could be steric in nature, *e.g.* in the hypothesis of the widening of the secondary hydroxy rim with subsequent narrowing of the primary one.

Experimental

β-Cyclodextrin (Serva) and ursodeoxycholic acid (Sigma) were used as received from the manufacturers; the inclusion complex was directly prepared in the 5 mm NMR tube. The solution of the complex was left to equilibrate for one day. ^{1}H and ^{13}C NMR spectra were obtained at 300 K (unless stated otherwise) using a Bruker AMX-400 WB spectrometer operating at 400.13 and 100.61 MHz, respectively, on 1.6×10^{-2} M D_2O solutions. Digital resolution was ±0.1 Hz for ^{1}H NMR spectra and ±0.3 Hz for ^{13}C NMR spectra. δ values (ppm) refer to $SiMe_4$.

Two-dimensional NMR spectra were obtained through standard pulse sequences (Bruker software). Conditions for ROESY[10,11] phase-sensitive spectra *via* time-proportional phase incrementation (TPPI) were: presaturation of residual HDO signal during relaxation delay (1 s); mixing time = 250 ms; spin-lock field = 4000 Hz; spectral width = 10 ppm with 2048 complex points in $f2$; 1024 $t1$ values and 24 scans per $t1$ value. A sine function (SSB = 4) was applied in $f1$ and $f2$ before Fourier transformation.

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