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NMR Investigations of the Inclusion of Thyroxine and Derivatives in Natural Cyclodextrins

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Abstract. Thyroxine T4 and its derivatives (T3, T2) are very sparingly soluble in aqueous solutions even in the form of salts. In the presence of β or γ-cyclodextrins and in buffered basic solution, their solubilities are increased by inclusion in the cavity. The inclusion of these hormones in cyclodextrins was investigated by ^{1}H-NMR in order to derive the influence of the number and position of the iodine atoms, and of the ionization state of the phenol group on the inclusion geometries.

Key words: cyclodextrins β and γ, thyroxine, thyroid hormones, inclusion complex, NMR.

1. Introduction

It is well known that cyclodextrins (CDs) can form inclusion compounds in aqueous solution with a variety of hydrophobic molecules [1]. The potential use of natural cyclodextrins and of synthetic derivatives has been extensively studied in order to improve certain properties of drugs such as solubility, stability and bioavailability [2]. CD complexes are in equilibrium with guest and host molecules in water, the degree of the dissociation being dependent on the magnitude of the stability constant [3]. This property of CD complexes is a desirable quality, because the dissociation leads to free CD and drug at the absorption site, and only the drug in the free form enters into systemic circulation. In this paper, we report on the study of the inclusion of L-thyroxine (T4) and its derivatives (T3, T2) in cyclodextrins. These molecules (Figure 1) are thyroid hormones of considerable importance for the metabolism of iodine and their physiological activities are well defined [4]. Two important sets of properties can be attributed to these hormones. During growth and development of either animals or humans, thyroid hormones play a critical role, especially in brain development since its absence leads to irreversible mental retardation (cretinism) which is accompanied by multiple morphological alterations. In the adult, thyroid hormones exert a major influence in maintaining

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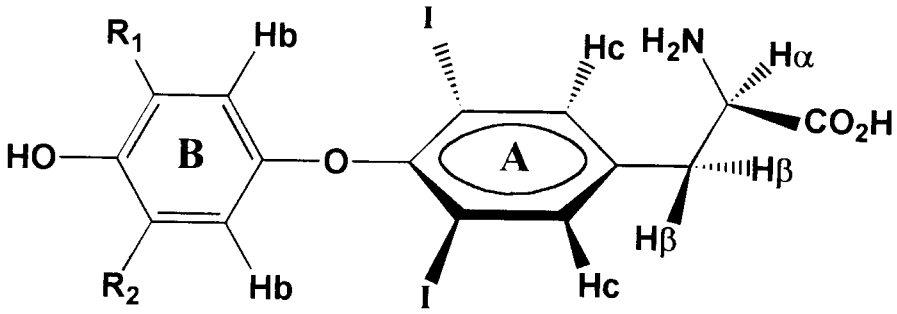

R1= R2= Ha: **T2**
R1= I, R2= Ha: **T3**
R1= R2= I: **T4**

Figure 1. Molecular structures of the thyroid hormones T2, T3 and T4.

metabolic homeostasis, thus acting on the vascular system by direct and indirect actions.

The solubility of these molecules strongly depends upon pH and the ionization state of the phenol group [5], with pKa values [4] of 9.8, 8.4 and 6.5 for T2, T3 and T4, respectively, according to the number of iodine atoms. It should be noted that the pKa values of the acid and amino groups were found in the literature [4] to be 2.2 and 10.2 respectively. However, all these compounds remain very sparingly soluble in aqueous solutions, even as sodium salts at alkaline pH [6].

The inclusion of Thyroxine in γ-CD has been reported to improve solubility, stability and membrane permeation properties [7]. However, no direct evidence is presented to support the existence of true inclusion complexes. A complete characterization in terms of stability and molecular conformation should contribute to a better understanding of the therapeutic properties of these complexes.

We investigated the inclusion process of thyroid hormones T2, T3 and T4 into the natural cyclodextrins: α-CD, β-CD or γ-CD. For this purpose, ^{1}H-NMR is used to prove the inclusion process and to afford a complete characterization of the complexes in aqueous solution. It is of interest to study the pH dependence of the stability and the molecular geometry of the inclusion complex. Moreover, the size of L-thyroxine and its derivatives strongly depends on the number of the bulky iodine substituents. For this reason, the inclusion process should be affected by the size of the cavity and by the degree of substitution of the L-thyronine core. We wish to demonstrate here that the use of cyclodextrins can strongly improve the solubility of thyroid hormones and this process will be demonstrated using ^{1}H-NMR. A crude model of the structure of the inclusion compounds is presented as derived from NMR data.

2. Experimental

Materials. L-thyroxine sodium salt (T4), 3,3′,5-triiodo-L-thyronine sodium salt (T3), and 3,5-diiodo-L-thyronine (T2) were obtained from Fluka AG. β-Cyclodextrin and α, γ-cyclodextrin were gifts from Roquette (France) and Wacker (Germany), respectively. Carbonate buffer (0.05 mol L^{-1}, pH 9.98) was obtained by dissolving 220 mg of anhydrous $NaHCO_3$ and 271 mg of anhydrous Na_2CO_3 in 100 mL of ultrapure water (Millipore system). pH was measured at room temperature. Phosphate buffer (0.05 mol L^{-1}, pH 7.2) was obtained by dissolving 355 mg of anhydrous Na_2HPO_4 and 300 mg of anhydrous Na_2HPO_4 in 100 mL of ultrapure water and the pH was adjusted to 7.2.

Preparation of Inclusion Complexes. The potential for cyclodextrins to improve the solubility in water of thyroid hormones was estimated using preliminary assays. The CD/drug mixtures were prepared by mixing accurately weighted quantities in the appropriate buffer, the final concentration of CDs and hormones being 2.5, 10 or 15 mmol L^{-1} respectively. All samples were homogeneized in an ultrasonic bath for 30 min at 30 °C, filtered and freeze-dried. After redissolution in deuterium oxide (Euriso-Top, France), they were freeze-dried again. The solubility of the guest compounds was derived from the integration of ^{1}H-NMR signals. The results obtained at 25 °C in carbonate buffer are displayed in Table II.

For example, the maximum solubility value of T2 in phosphate buffer (pH 7.2), in the case of β-CD and γ-CD complexes were obtained by integration of the aromatic signals of T2, and compared with the integration values of H1 of cyclodextrin. The maximum concentration of T2 obtained with the β-CD complex was 1.5 mmol L^{-1} and with the γ-CD complex 1.2 mmol L^{-1} (for a 10 mmol L^{-1} concentration in CD).

Method. ^{1}H NMR experiments were performed at 500 MHz using a Bruker DRX500 spectrometer. In all cases, the samples were prepared in deuterium oxide (Euriso-Top, Saclay, France) and measurements were performed at 298 K under careful temperature regulation. Unless indicated otherwise, all solutions in D_2O were adjusted to pH 9.98 (uncorrected meter reading). The length of the 90° pulse was ca. 7 μs. 1D NMR spectra were collected using 16 K data points. Chemical shifts are given relative to external tetramethylsilane (TMS = 0 ppm) and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. ROESY experiments [8] were obtained using the pulse program available from the Bruker library using a 300 ms spin-lock time. These bidimensional experiments were acquired using 2 K data points and 256 time increments. The phase sensitive (TPPI) sequence [9] was used and processing resulted in a 1 K $\times$ 1 K (real-real) matrix. Details concerning experimental conditions are given in the figure captions. All NMR data were processed and plotted using the UXNMR program (Bruker Analytische Messtechnik) on an INDY workstation (Silicon graphics).

Table I. Solubility (mol L^{-1}) at 25 °C of thyroid hormones alone versus pH

	T2	T3	T4 [Ref. 6]
pH 7.2	6×10^{-5}	4×10^{-5}	3×10^{-5}
pH 9.98	7×10^{-4}	2×10^{-4}	8×10^{-5}

Molecular Drawings. Molecular models were obtained using Chem3D and Weblab Viewer softwares, running on a PC station. The two-component inclusion complexes were constructed by manually docking the guest molecules into the cyclodextrin cavities. The docking energy was minimized and all atoms were kept at internuclear distances larger than the sum of their van der Waals radii. For the sake of the clarity, inclusion complexes were displayed by "ball and stick" representations for the guest molecules, and by the solvent-accessible surface for the cyclodextrin components, respectively.

3. Results and Discussion

3.1. SOLUBILITY OF THYROID HORMONES

Thyroid hormones T2, T3 and T4 are very sparingly soluble in aqueous solution even at alkaline pH. The solubility at 25 °C of L-Thyroxine (T4) is very well described in the literature [6]. The solubility in water at 25 °C has been determined for T2 and T3. The results are displayed in Table I. The solubility of T3 remains lower than 10^{-4} mol L^{-1} even at alkaline pH. T2 is the most soluble thyroid hormone.

The solubilization of thyroid hormones by α, β and γ-CD was determined as described in the experimental section. The following conclusions can be drawn from the detailed analysis of the experimental data.

It is observed that α-cyclodextrin does not improve the solubility of any thyroid hormones at neutral pH. This observation implies that no formation of inclusion complex occurs. On the other hand, the maximum concentration of T2 obtained with the β-CD complex was 1.5 mmol L^{-1} and with the γ-CD complex 1.2 mmol L^{-1} (for a 10 mmol L^{-1} concentration in CDs). The solubility of T2 is increased by a 2 fold factor in the presence of 8 equivalents of β-CD or γ-CD. These observations suggest the possible formation of weak inclusion complexes with T2 at pH 7.2. Trials to improve the solubility of T3 or T4 by the presence of β or γ-CD at pH 7.2 failed.

On the other hand, at pH 9.98, with the exception of T4 with β-cyclodextrin, the solubility of all thyroid hormones is strongly increased, reaching 8.5 mM for T2 in the presence of 2 equivalents of γ-cyclodextrin (Table II). For example, in the presence of 1.5 mmol L^{-1} of γ-CD, the solubility of T4 is increased by a 50 fold factor. These results represent the first clue for the formation of inclusion complexes since

Table II. Solubility (mol L^{-1}) at 25 °C of thyroid hormones at pH 9.98 in the presence of the pertinent cyclodextrin

Cyclodextrins	T2	T3	T4
α-CD 2.5×10^{-3}mol L^{-1}	7×10^{-4}	2×10^{-4}	8×10^{-5}
β-CD 2.5×10^{-3}mol L^{-1}	2.2×10^{-3}	1.9×10^{-3}	2×10^{-4}
β-CD 10×10^{-3}mol L^{-1}	4.2×10^{-3}	3.1×10^{-3}	4×10^{-4}
β-CD 15×10^{-3}mol L^{-1}	5.6×10^{-3}	4.3×10^{-3}	4×10^{-4}
γ-CD 2.5×10^{-3}mol L^{-1}	2.3×10^{-3}	2.2×10^{-3}	2.4×10^{-3}
γ-CD 10×10^{-3}mol L^{-1}	4.5×10^{-3}	3.8×10^{-3}	3.6×10^{-3}
γ-CD 15×10^{-3}mol L^{-1}	8.2×10^{-3}	5.5×10^{-3}	4.2×10^{-3}

the guest molecules are in the salt form. It should be noted that T4 is substituted by four iodine atoms and a first explanation for the lack of complexation of T4 with β-CD at pH 9.98 involves steric hindrance effects. The addition of α-CD does not increase the solubility of any thyroid hormone even at pH 9.98 since the small cavity of α-CD cannot accommodate even the smallest hormones. These observed results will be tentatively explained in the following section in the light of detailed NMR analysis.

3.2. NMR INVESTIGATIONS

In the first step, ^{1}H-NMR spectroscopy was used to confirm the formation of inclusion complexes and to estimate the possible effects of the number and the position of the iodine atoms at pH 7.2 and 9.98 (phosphate and carbonate buffers, respectively). It must be borne in mind that all data presented here were obtained in buffered solution.

First, we tried to obtain an inclusion complex at pH 7.2. Inclusion of the thyroid hormone T2 in β-CD is evidenced by modifications of the ^{1}H-NMR spectrum of the host molecule [10]. The 500 MHz NMR spectra of β-CD and of the mixture are displayed in Figure 2. Under these conditions, only shifts of the signals were observed. No new peak appeared which could be assigned to the pure complex. This observation implies that the complexation is a dynamic process, the included drug being in the fast exchange regime (relative to the NMR time scale) between the free and bound states. The largest shifts are observed for protons H3 and H5 located in the β-CD cavity. These upfield shifts are induced by ring current effects of the included phenyl group and are considered as a proof for the formation of an inclusion complex [3, 10].

Attempts to obtain inclusion complexes with β-CD and the other thyroid hormones T3 and T4 at pH 7.2 failed since no detectable shifts were observed with T3 and T4 under the same conditions. In the same way, we could not observe any

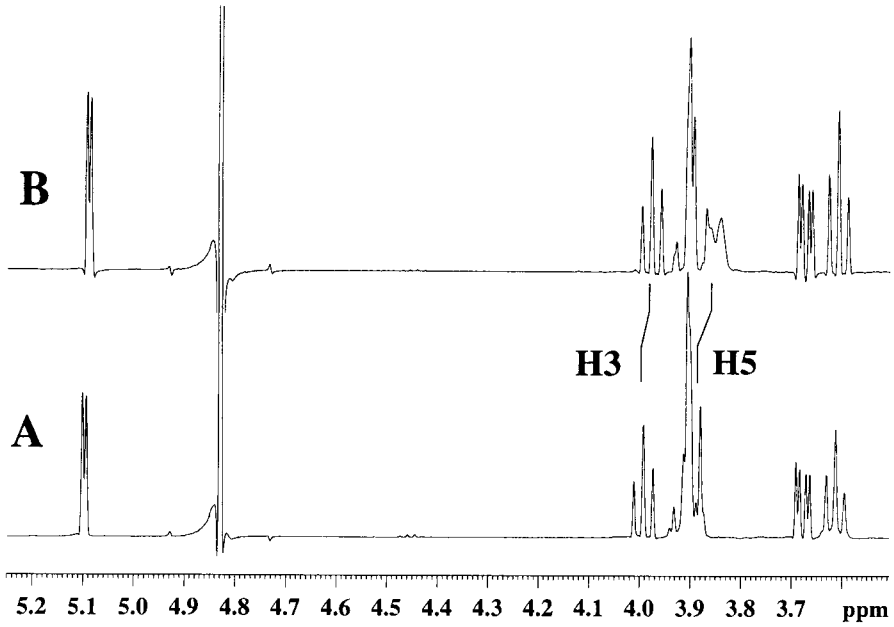

Figure 2. Partial ^{1}H-NMR spectra (500 MHz, D_2O, pH 7.2, 298K) of β-CD 2.5 mmol L^{-1} alone (A) and in the presence of 1.25 mmol L^{-1} T2 (B).

inclusion process between α-CD with T2, T3 and T4 and between γ-CD with T3 and T4 at pH 7.2. This fully agrees with the solubilization experiments. A small increase of solubility of T2 was noted with γ-CD but no detectable shifts were observed in the ^{1}H-NMR spectra. These observations lead us to conclude that, in this case, the inclusion process is unfavourable. The presented data clearly indicate that the state of the phenol group plays a key role in the stabilization of the complexes most probably through interactions between the phenolate and the hydroxyl moieties of the host.

The same ^{1}H-NMR experiments have been performed in buffer solution at pH 9.98. Figure 3 shows partial spectra of β-CD alone and in the presence of T2, T3 and T4 at pH 9.98.

Addition of T2 and T3 induced shifts in the signals originating from protons H3 and H5 of β-CD. It should be noted that addition of T4 did not induce significant shifts in the signals of β-CD. These results confirm the absence of complexation of T4 by β-CD and the formation of inclusion complexes between T2 and T3 with β-CD as also demonstrated by solubilization assays.

A more careful analysis of the data shows that under strictly identical conditions, T2 induces larger upfield shifts in the signals of H3 and H5 of β-CD than T3. The downfield shift of the H3 proton could be explained by the orientation of the B ring of T3 in the cavity since the bulky iodine atom strongly modifies the geometry of the inclusion complex. The ring is no longer along the symmetry axis

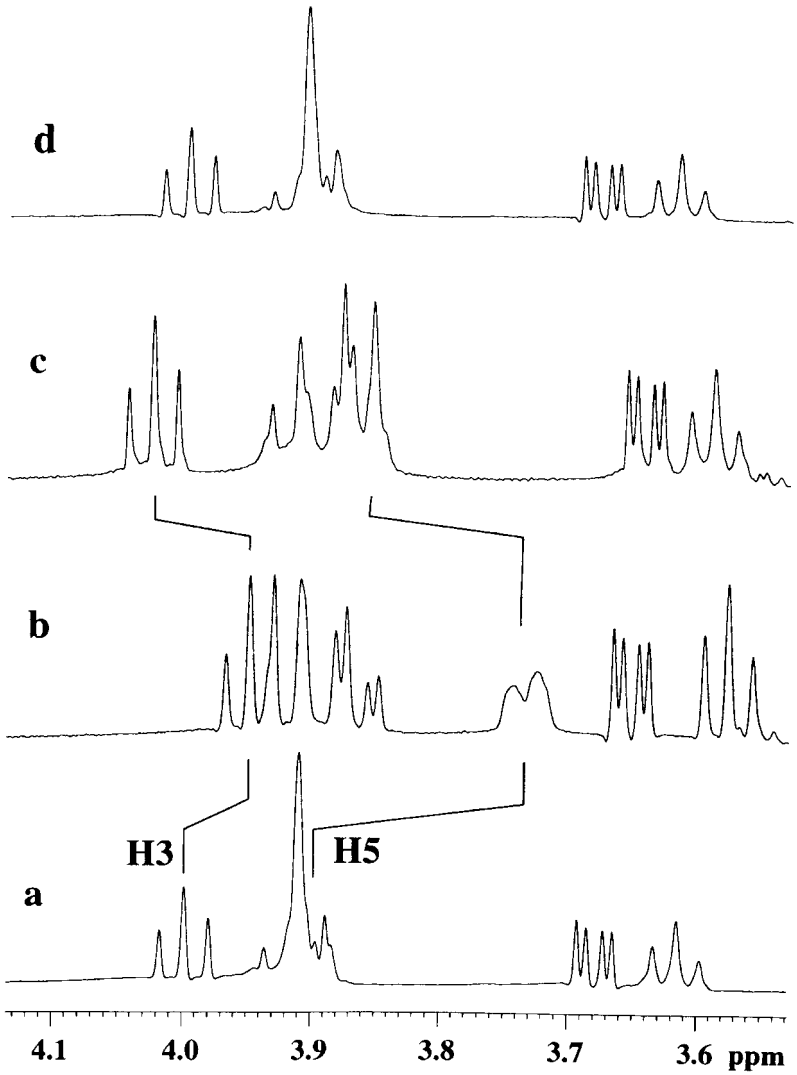

Figure 3. Partial ^{1}H-NMR spectra (500 MHz, D_2O, pH 9.98, 298K) of β-CD 2.5 mmol L^{-1} alone (a) and in presence of T2 2.5 mmol L^{-1} (b) and in the presence of T3 2.5 mmol L^{-1} (c) and in presence of T4 2.5 mmol L^{-1} (d).

of β-CD and the H3 proton is not located in the positive anisotropic part of the ring as shown by ROESY experiments and molecular models derived therefrom.

The observed differences are probably due to the weaker affinity for T3 as compared to T2. At this point, it should be assumed that the affinity of β-CD for thyroid hormones (T4 $\ll$ T3 $\sim$T2) decreases rapidly as the number of iodine atoms increases according to pure steric effects. The same detailed NMR study has been performed using γ-CD as host. Comparison of the ^{1}H-NMR spectra of γ-CD in

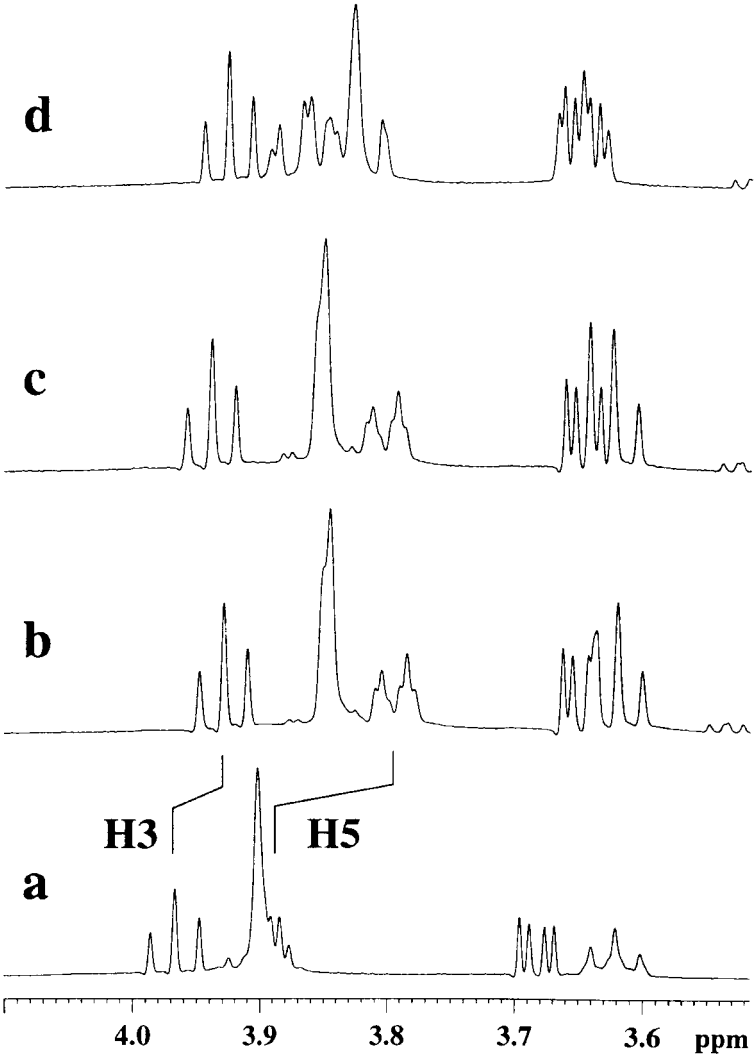

Figure 4. Partial ^{1}H-NMR spectra (500 MHz, D_2O, pH 9.98, 298K) of γ-CD (2.5 mmol L^{-1}) alone (a) and in presence of T2 (2.5 mmol L^{-1}) (b), in the presence of T3 (2.5 mmol L^{-1}) (c) and in the presence of T4 (2.5 mmol L^{-1}) (d).

the absence and in the presence of T2, T3 or T4 (Figure 4) clearly indicates the formation of inclusion complexes at alkaline pH.

However, we could observe that addition of T2 and T3 induced the same upfield shifts of the signals of H3 and H5 of γ-CD. In contrast weaker shifts are experienced in the presence of T4 under the same conditions. It appears that the affinity of γ-CD is similar for T2 and T3 and weaker for T4. γ-CD is therefore shown to form inclusion complexes with all thyroid hormones even if their affinities are weaker for the most bulky guest, since it is well known that γ-CD has considerable potential

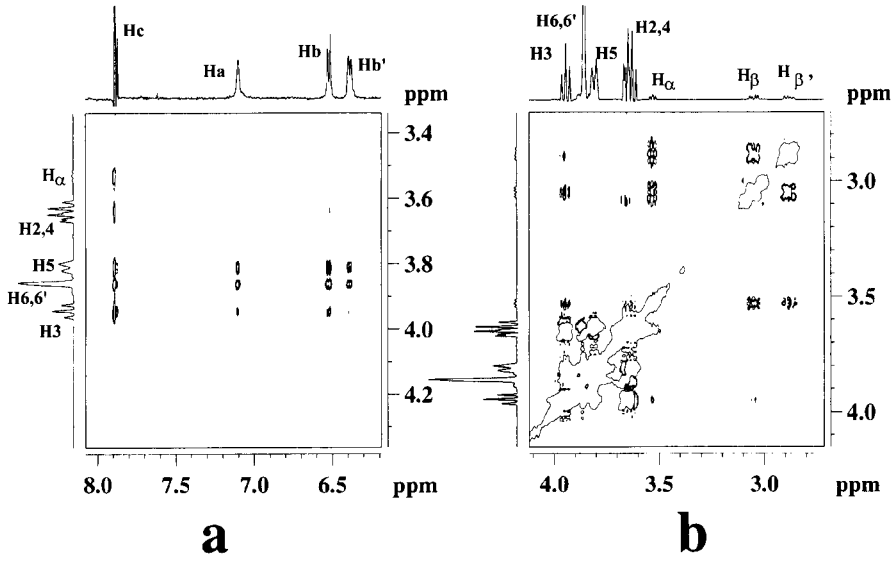

Figure 5. Partial contour plots of ROESY experiment (spinlock time: 300 ms) performed at 500 MHz on the inclusion complex γ-CD/T3 (2.5 mmol L^{-1}) in D_2O, pH 9.98 at 298K: areas of aromatic protons (a) and aliphatic protons (b).

inclusion capacity owing to its flexibility and ability to host large hydrophobic molecules [11]. Finally, comparison of the ^{1}H-NMR spectra of α-CD (data not shown) in the absence and in the presence of T2, T3 and T4 clearly shows that no inclusion complex occurred at pH 9.98 with α-CD.

More detailed indications concerning the geometry of these inclusion complexes can be derived from the evidence of the spatial proximities between protons of the host and guest molecules. This can be achieved by investigations of dipolar interactions using 2D ROESY experiments [8] since it was shown that this provides the most sensitive approach to the structural analysis of inclusion complexes with CD [12] in solution. As found in many other situations regarding inclusion complexes in water [12, 13], the 300 ms mixing time was selected to provide reliable dipolar cross-peaks with a minimal contribution of scalar transfer.

A typical example is displayed in Figure 5 for the γ-CD/T3 inclusion complex. All non-diagonal peaks are indicative of spatial proximities between protons. Dipolar contacts are observed between protons H3 and H5 of cyclodextrin with all aromatic protons of the thyroxine T3. Weaker interactions are observed between proton Hc of T3 and protons H2 and H4 of γ-CD and between protons Hα and Hβ of T3 and proton H3 of γ-CD. This careful analysis, especially of peaks involving the side chain of this hormone, suggests that the inclusion of the guest molecule takes place from the wide secondary hydroxy group side and that most of T3 is located inside the large cavity of the γ-CD. These results suggest that a 1 : 1 complex between γ-CD and guest T3 was likely formed.

Table III. Interactions observed between protons of thyroid hormones T2, T3 and T4 and protons of β-CD and γ-CD, during ROESY experiments performed on the inclusion complexes (2.5 mmol L^{-1}, D_2O, pH 9.98, 298 K)

	βCD			γCD		
	H3	H5	H6	H3	H5	H6
T2,Ha	+++	+++	++	++	+++	+++
T2,Hb	+++	+++	++	++	+++	+++
T2,Hc	+	–	–	+++	+++	++
T2,Hβ	–	–	–	++	–	–
T2,Hβ'	–	–	–	–	–	–
T2,Hα	–	–	–	++	–	–
T3,Ha	+++	+++	+++	+	++	++
T3,Hb$'$	++	+++	+++	+	+++	+++
T3,Hb	++	+	–	–	+++	+++
T3,Hc	++	–	–	+++	+++	+++
T3,Hβ	–	–	–	++	–	–
T3,Hβ'	–	–	–	–	–	–
T3,Hα	–	–	–	++	–	–
T4,Hb				+	+++	+++
T4,Hc	No inclusion complex			+++	++	++
T4,Hβ'				+	–	–
T4,Hβ				+	–	–
T4,Hα				–	–	–

+++ Strong interaction.
++ Medium interaction.
+ Weak interaction.
– No interaction.

The same NMR experiments have been performed under strictly identical experimental conditions with the following inclusion complexes: β-CD/T3, β-CD/ T2, γ-CD/T2 and γ-CD/T4. The main results are reported in Table III. Comparison of the interactions obtained for the inclusion complexes β-CD/T3 and γ-CD/T3 leads to the following observations. First, the number and the strength of these interactions are higher in the γ-CD/T3 inclusion complex than in the β-CD/T3 inclusion complex. Second, only the phenol group of T3 (ring B) seems to be implicated in the inclusion process with β-CD in contrast to γ-CD for which rings A and B are located inside the cavity. These results show that T3 penetrates deeper inside the largest cavity of γ-CD than inside that of β-CD. These conclusions are consistent with the literature [5].

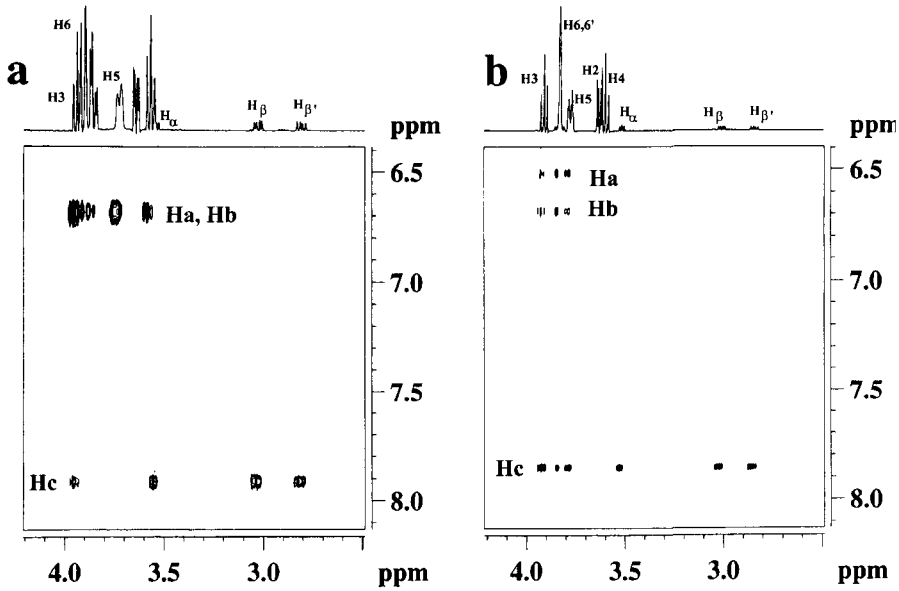

Figure 6. Partial contour plots of ROESY experiment (spinlock: 300 ms, attenuation 17 dB) performed at 500 MHz on the inclusion complex β-CD/T2 (a) and on the inclusion complex γ-CD/T2 (b) (2.5 mmol L^{-1}) in D_2O, pH 9.98 at 298 K. Horizontal scale: Cyclodextrin protons and aliphatic T2 protons region; Vertical scale: aromatic region.

The same careful analysis of the spatial proximities between protons of T2 and of βCD or γCD has been performed as shown in Figure 6. It appears that this guest forms inclusion complexes with almost the same spatial geometries as T3.

The difference of shifts experienced by protons Ha and Hb of the guest molecule upon complex formation is very informative. Indeed, protons Ha and Hb are almost isochronous for the β-CD/T2 inclusion complex and highly inequivalent for the γ-CD/T2 inclusion complex. These shifts of the aromatic protons of T2 are due to variations of the local polarity and therefore to different positions of this aromatic moiety inside the cavity of the considered cyclodextrin.

Morever, it should be kept in mind that attempts to form an inclusion complex between T4 and β-CD failed. Conversely, as shown in Table III, dipolar contacts (indicating spatial proximities) are observed between proton Hb (ring B) and Hc (ring A) of T4 and protons H3, H5 and H6 of γ-CD. In contrast to the inclusion complexes γ-CD/T2 and γ-CD/T3, very weak interactions involved protons Hβ of the aliphatic chain of T4 and proton H3 of γ-CD. These results suggest that although the presence of four iodine atoms induces strong steric hindrance, the inclusion of T4 takes place from the wider side as with the smaller guests T2 and T3, but that the aliphatic chain is less implicated in the inclusion process.

To conclude this part, the results obtained by ROESY experiments are in complete agreement with those derived by ^{1}H-NMR at pH 9.98 and can be summarized as follows:

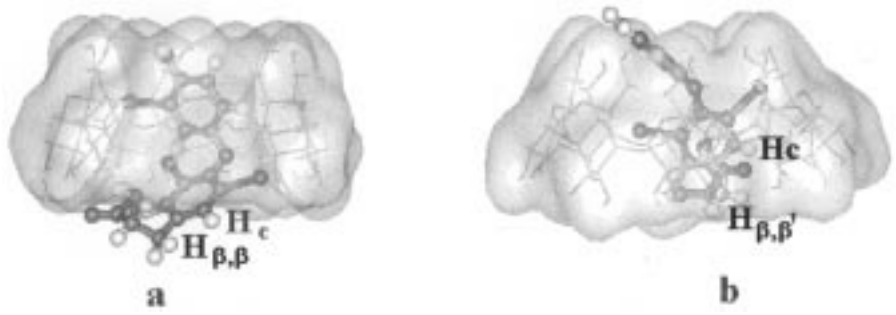

Figure 7. Proposed structures for the β-CD/T3 inclusion complex (a) and γ-CD/T3 inclusion complex (b).

- T2 and T3 form inclusion complexes with β-CD with similar affinity and spatial geometry. The phenol group (ring B) is the most implicated in the inclusion complex, the aliphatic chain remaining outside of the cavity of β-CD. With T4, no inclusion complex can be observed in the presence of β-CD owing to a too narrow cavity.
- with γ-CD, we have obtained inclusion complexes with all three guests. The affinity and the spatial geometry of γ-CD/T2 and γ-CD/T3 appear quite similar. Almost the whole molecule fits inside the cavity of γ-CD, the aliphatic chain being involved in interaction with the secondary hydroxyl side. The presence of four bulky iodine atoms does not preclude the formation of the inclusion complex γ-CD/T4 although a decrease in affinity is observed.

In order to evaluate the possibility of the formation of an inclusion complex, molecular modelling was used to derive a realistic model for the complexes. Models of the inclusion complex can be suggested taking into account the observed spatial proximities in aqueous solution. Figure 7 displays proposed probable structures for the βCD/T3 and γCD/T3 inclusion complexes.

It should be emphasized that all spatial proximities (internuclear distance lower than 5 Å) defined in these models are observed as cross-peaks in the ROESY experiments. The aromatic ring B docks within the β-CD or γ-CD cavity from the secondary hydroxyl rim of the host. With β-CD, the aromatic ring A is then partially located outside the cavity in agreement with the ROESY experiment since only one weak dipolar interaction has been observed between proton Hc and proton H3 of β-CD. As illustrated in the model, the aliphatic chain is located outside the cavity of β-CD.

Conversely, with γ-CD, the penetration of the guest molecule is deeper and ring B is exposed to the primary hydroxyl rim of the host. This hypothesis is supported by the cross-peaks observed between protons Ha, Hb and even Hc and proton H6 of γ-CD. The aliphatic chain is partially located inside the cavity in agreement with the ROESY experiment.

These models clearly demonstrate that the size of the host cavity influences the geometry of the inclusion complexes.

4. Conclusion

No inclusion complexes with Thyroid hormones T2, T3 or T4 could be obtained with α-CD since its cavity appears to be too narrow. Thyroid hormones T2 and T3 are included in β and γ-cyclodextrins and form inclusion complexes with 1 : 1 stoichiometry at alkaline pH. The more bulky molecule T4 was found to include only in the cavity of γ-CD, the largest and the most flexible natural cyclodextrin, in full agreement with the data presented in Reference 7. The inclusion process, when present, induces a very large increase in the solubility of these hormones in water and may represent a way to improve the oral administration of these drugs since an appropriate thyroid hormone replacement therapy is the only way of treating either congenital or surgical hypothyroidism.

It is shown here that a selection of the most convenient cyclodextrin allows improvement of the solubilization of any thyroid hormone. NMR ROESY experiments allow realistic geometrical models to be drawn for the inclusion complexes in solution, in agreement with NMR data. The slight differences in the proposed positions of rings A and B of the Thyroid hormones included in the β-CD or γ-CD cavities reflect the effect of the number of iodine substituents. The guests in the γ-CD complexes are more deeply inserted into the cavity than is the case for β-CD. It seems that the stability of the inclusion complexes of β-CD and γ-CD with T2, T3, or T4 depends on the size of the guests, on the nature of the CD and on the ionization of the phenolic hydroxyl groups (ring B).

References

1. (a) D. Duchêne (ed.), *New Trends in Cyclodextrins and Their Derivatives*, Editions de Santé, Paris, (1991). (b) K. Uekama and F. Hirayama: in C.G. Wermuth (ed.), *The Practice of Medicinal Chemistry*, Academic Press, London, 793 (1996).
2. W. Saenger: *Angew. Chem. Int. Ed. Engl.* **19**, 344 (1980).
3. F. Djedaïni, S.Z. Lin, B. Perly, and D. Wouessidjewe: *J. Pharm. Sci.* **79**, 643 (1990).
4. R.M.C. Dawson, D.C. Elliott, W.H. Elliott, and K.M. Jones: *Data for Biochemical Research*, Second Edition, Clarendon Press, Oxford (1969).
5. Y. Inoue, T. Okuda, Y. Miayata, and R. Chujo: *Carbohydr. Res.* **125**, 65 (1984).
6. H.E. Evert: *J. Phys. Chem.* **64**, 478 (1960).
7. W. Fisher and D. Bracher: *PCT Int. Appl.* WO 9719703 A2 970605 (1997).
8. A. Bax and D.G. Davies: *J. Magn. Reson.* **65**, 355 (1985).
9. G. Bodenhausen, H. Kogler, and R.R. Ernst: *J. Magn. Reson.* **58**, 370 (1984).
10. F. Djedaïni and B. Perly: *Magn. Reson. Chem* **28**, 372 (1990).
11. F. Djedaïni and B. Perly: *J. Mol. Struct.* **239**, 161 (1990).
12. V. Laine, A. Coste-Sarguet, A. Gadelle, J. Defaye, B. Perly, and F. Djedaïni-Pilard: *J. Chem. Soc. Perkin Trans 2*, 1479 (1995).
13. F. Djedaïni-Pilard, N. Azaroual-Bellanger, M. Gosnat, D. Vernet, and B. Perly: *J. Chem. Soc. Perkin Trans 2*, 723 (1995).