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NMR Studies on the interaction between (−)-epigallocatechin gallate and cyclodextrins, free and bonded to silica gels

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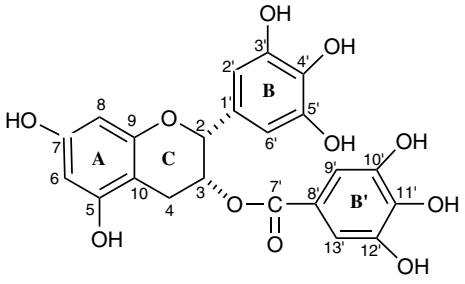
Abstract—The interaction between (−)-epigallocatechin-3-gallate (EGCG) and β- or γ-cyclodextrin (CD), in free solution and bonded to silica beads, has been studied by ^{1}H HR-MAS NMR spectroscopy. The chromatographic retardation of EGCG on columns packed with CD-silica beads was shown to be due to the interaction of EGCG with the CD ligands because no nonspecific interaction with the silica gel could be observed. EGCG forms a tighter complex with β-CD than with γ-CD and NMR data obtained from hydroxy protons together with MM2 calculations suggest that for β-CD intermolecular hydrogen bonding, in addition to hydrophobic interaction, stabilizes the complex.

Keywords: Cyclodextrins; Epigallocatechin gallate; HR-MAS NMR; Silica beads

1. Introduction

Catechins, the major group of polyphenols in green tea, have attracted attention due to their antioxidants activity[1,2] and bacteria[3,4] and cancer[5] inhibiting properties. The most important catechin seems to be (−)-epigallocatechin-3-gallate (EGCG) (Scheme 1) that may also have antiretroviral properties.[6] These compounds are most commonly analyzed by liquid chromatography, and cyclodextrin-bonded stationary phases have shown to be valuable alternatives for their separation and purification.[7–10]



Scheme 1. Structure of EGCG.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucose units connected by α-1,4-glycosidic linkages. With a shape like a truncated cone, they form a hydrophobic cavity, capable of creating inclusion complexes with various molecules by incorporating them into the cavity. The ability of a CD to form an inclusion complex with a guest molecule is a function of steric as well as thermodynamic factors. The driving forces for complex formation are attributed to the removal of water molecules from the hydrophobic cavity and the formation of van der Waals, hydrophobic and hydrogen bond interactions.[11,12]

Even if the technique of chromatography is well known, there is often inadequate knowledge of the mechanisms involved in the chromatographic separation. Studies of the interactions between the solute and the stationary phase on a molecular level are therefore

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crucial for the design of more efficient chromatographic media. NMR spectroscopy is the method of choice to monitor these intermolecular interactions since several NMR parameters will change in a characteristic manner upon binding. High-resolution magic-angle spinning (HR-MAS) NMR allows a direct study of the interactions between analytes and immobilized chromatographic ligands. As NMR experiments can be performed under conditions similar to those used during the chromatographic separation process, they allow a direct comparison of the data obtained. HR-MAS NMR has been shown to be a useful technique to study molecular interactions between solutes and silica based stationary phases, allowing the acquisition of spectra with a high resolution.[13–15]

Recently the chromatographic retention of EGCG on oligo-β-cyclodextrin coupled to agarose gel media was correlated to association constants obtained for EGCG and free β-CD using ^{1}H solution state NMR.[16] The retention was shown to be mainly due to the presence of the β-CD ligand. Nonspecific adsorption was not found to contribute to the retention of the solute. As the β-CD and EGCG were both in solution in this NMR study a direct comparison to their chromatographic behaviour was difficult to make. In the current study ^{1}H HR-MAS NMR was used to monitor the interaction between EGCG and β- and γ-CD bonded silica beads (CYCLOBOND I and II stationary phases). For comparison, the interaction between EGCG and free β- and γ-CD was also analyzed using liquid state HR NMR. Hydrophobic interaction between the solute and the immobilized ligand is expected to dominate with water as a solvent. However, it has been proposed that the observed chromatographic retention might be due to hydrogen bond formation between β-CD and EGCG rather than to hydrophobic interaction.[17] The existence of intermolecular hydrogen bonds between EGCG and β-CD has also been suggested based on AM1 calculations,[18] but no direct experimental proofs are yet available. To answer the question as to what extent the retention is controlled by hydrophobic or hydrophilic interaction, the exchangeable hydroxy protons of EGCG and of the CDs have been studied by ^{1}H NMR spectroscopy as well.

2. Results and discussion

The possible structure of the inclusion complexes was studied from changes in chemical shifts and linewidths of the signals of the exchangeable OH and the nonexchangeable CH protons and from intermolecular ROEs. The temperature coefficients of the hydroxy proton signals were also measured. To avoid self-association of EGCG, reported to occur for catechins at high concentrations,[19,20] 5 mM solutions of CD and EGCG were used.

2.1. Complex formation between EGCG and free α-CD

No significant changes in the chemical shifts of the proton signals of EGCG or α-CD and no intermolecular ROEs between the two compounds in a 1:1 mixture were observed indicating that no inclusion complex was formed. The inner α-CD cavity is too small (5.7 Å) to allow penetration of the substituted aromatic rings, and this system was not further investigated.

2.2. Complex formation between EGCG and β-CD free and bonded to the silica beads

2.2.1. Coupling amount. The 1D proton NMR spectrum of the insoluble β-CD substituted silica gel sample (CYCLOBOND I) swollen in D_2O and recorded using a 4 mm HR-MAS probe head is shown in Figure 1. For comparison, the spectrum of the same silica gel recorded with a conventional 5 mm HR probe is also shown. With the HR-MAS probe, the NMR spectrum of the gel has spectral resolution similar to that usually obtained for homogeneous liquid solution. The amount of β-CD coupled to the silica beads was determined from HR-MAS NMR spectra by analysing the signals of β-CD relative to that of the internal standard sodium 3-trimethylsilyl[2,2,3,3-d_4]propionate (TSP-d_4) (see Section 4). TSP-d_4 is commonly used as an internal refer-

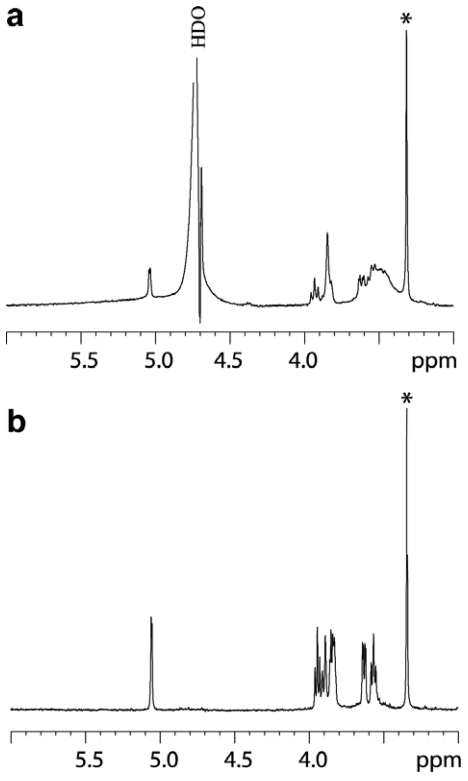

Figure 1. 1D ^{1}H NMR Spectra of the β-CD gel recorded at 600 MHz (D_2O, 25 °C) in (a) a 5 mm HR probe and (b) a 4 mm HR-MAS probe (spinning rate: 4 kHz). ∗: Spacer.

ence for aqueous solution, as well as for quantitation since it does not interact with the sample and it is not degraded during the NMR experiment. A coupling amount of 90–95 μmol of β-CD per gram gel was obtained, in good agreement with the data provided by the supplier.

2.2.2. Nonspecific interactions. The occurrence of non-specific interaction was investigated by recording HR-MAS NMR of EGCG in the presence of silica gel without bonded β-CD. The NMR spectra of EGCG were identical to the NMR spectra of EGCG alone suggesting that no significant binding of EGCG to the silica gel occurs. Thus, all changes observed are due to the interaction between EGCG and β-CD.

2.2.3. Complexation in D_2O. The ^1H NMR spectra of EGCG/free β-CD in D_2O obtained with a 5 mm HR probe and of EGCG/CYCLOBOND I obtained with a 4 mm HR-MAS probe were very similar (Fig. 2). The observed differences are discussed below. During the course of this work, a NMR study on the structure of the inclusion complex formed in D_2O solution between free β-CD and EGCG was published.[18] Our results with free β-CD are in good agreement with those reported, and thus this paper mainly focuses on data obtained with immobilized β-CD.

2.2.3.1. Line broadening. For a 1:1 solution, only one set of NMR signals was observed for both β-CD and EGCG indicating a rapid exchange on the NMR time scale. However, most of the proton signals of EGCG were broadened. The broadening of the resonances may be attributed to the restricted motion of the protons upon inclusion in the CD cavity. The EGCG proton signals that were not broadened, H9′, H13′ and H4α, are probably not located inside the CD cavity. When the temperature was increased, which causes a higher mobility or a decrease in the complex formation, the broad signals became sharper with the exception of those from H-6 and H-8. These two protons easily exchange with deuterium in D_2O. As noticed by Ishizu et al., the exchange is slower in the presence of β-CD than for EGCG alone.[18] This finding suggested that H-6 and H-8 were difficult to exchange to deuterium due to inclusion of the A ring into the β-CD cavity. The exchange rate of H-6 and H-8, in EGCG alone and in the complex, was measured by monitoring the sum of the integration values of the two proton signals, and no distinction between the two signals was made.[18] In the 85% H_2O/15% $(CD_3)_2CO$ and 95% H_2O/5% D_2O solvent systems, where deuterium exchange is not possible, H-6 appeared as a sharp signal, while H-8 was broader. Upon decreasing the temperature, H-8 as well as H-2, H-3 and H-4β broadened while H-6 remained a sharp

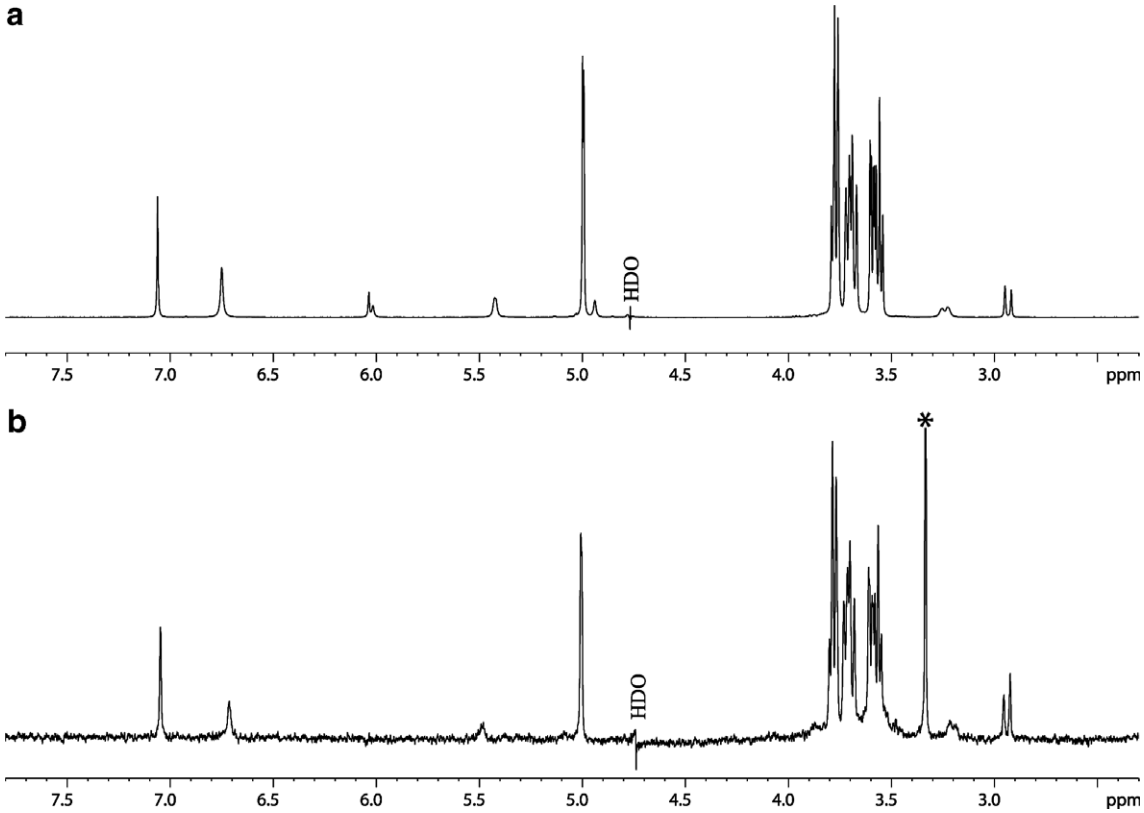

Figure 2. 1D ^1H NMR Spectra of sample in D_2O and at 25 °C containing (a) free β-CD/EGCG (1:1) recorded with a 5 mm probe and (b) β-CD gel/EGCG (1:1) recorded with an HR-MAS probe. ∗: Spacer.

signal. These observations indicate that H-8 is included in the hydrophobic CD cavity while H-6 is probably lying outside.

2.2.3.2. Chemical shifts. The addition of EGCG to a β-CD solution resulted in the shielding of H-3 and H-5, positioned on the inner surface of β-CD, and in the deshielding of H-1, H-2 and H-4 located on the outer surface of the torus (Table 2). The shielding is due to the ring current effect of the aromatic systems in EGCG and indicates that EGCG is entering the CD cavity. The EGCG protons were also affected by the presence of β-CD. H-2, H-3, H-6 and H-8 were shielded while H-4β and H-2′/H-6′ were deshielded (Table 1).

2.2.3.3. ROESY. ROESY experiments with mixing times 200 and 500 ms showed cross-peaks corresponding to strong ROEs between H-5 of β-CD and H-8 of EGCG and between H-3 of CD and H-2 and H-2′/H-6′ of EGCG. Medium ROEs were observed between H-3 of β-CD and H-3, H-4α,β and H-9′/H-13′ of EGCG. These results suggest that EGCG penetrates the CD cavity from the wide rim.

2.2.3.4. Comparison of interaction between EGCG and β-CD in free solution and β-CD bonded to silica beads. NMR experiments, run on the EGCG/β-CD bonded to silica beads directly after preparation of the sample, showed that complex formation was not yet at equilibrium as the chemical shifts of the signals were still changing. During the time necessary to reach equilibrium (~2 h), the H-6 and H-8 of EGCG were exchanging with deuterium and were therefore not visible in the proton NMR spectra (Fig. 2b). With CYCLOBOND I, EGCG must first diffuse into the pores of the matrix before it can bind to the ligand β-CD on the surface of the silica gel. In solution, the formation of inclusion complex is very rapid and the exchange of H-6 and H-8 with deuterium is slower (Fig. 2a). The chemical shift data and ROEs obtained with CYCLOBOND I were very similar to those obtained with free β-CD and indicate similar structures of inclusion complex. In the ROESY spectra of EGCG with CYCLOBOND I the H-6 and H-8 signals were not observed as the protons were exchanged by deuterium.

2.2.4. Complexation in 85% H_2O/15% $(CD_3)_2CO$: hydroxy protons. The aim of studying the hydroxy protons was to find an experimental evidence for the existence of hydrophilic interactions between β-CD and EGCG. Since the structure of the inclusion complex was the same in the gel and the free CD, the study was done in solution with free β-CD. In this way, inherent problem with observation of hydroxy protons in H_2O solution due to the acidity of the silica gel was avoided.

2.2.4.1. Hydroxy protons in β-CD and EGCG alone. The chemical shifts for the hydroxy proton signals of β-CD are given in Table 3 and these are in good agreement with the previously reported values.[21] For EGCG, six hydroxy proton resonances were observed (Fig. 3). Due to the lack of three bond couplings, the assignments could not be obtained from scalar connectivities to CH protons using COSY type experiments and were instead obtained from ROESY experiments

Table 1. ^{1}H NMR Chemical shifts of CH protons of EGCG alone (δ, ppm) and complexation induced shifts (CIS) when in complex with β-CD (CIS (β-CD)) and γ-CD (CIS (γ-CD)) at 25 °C

	H-2	H-3	H-4α	H-4β	H-6	H-8	H-2′,6′	H-9′,13′
δ	5.099	5.607	2.95	3.062	6.182	6.153	6.606	7.012
CIS (β-CD)	−0.159	−0.182	−0.016	0.179	−0.146	−0.137	0.144	0.049
CIS (γ-CD)	−0.025	−0.054	−0.021	0.019	−0.026	−0.033	0.040	0.017

Table 2. ^{1}H NMR Chemical shifts (δ, ppm) for CH protons of β-CD and γ-CD alone and their complexation induced shifts (CIS) when in complex with EGCG (25 °C)

		H-1	H-2	H-3	H-4	H-5	H-6
β-CD	δ	5.062	3.534	3.856	3.476	3.748	3.767
	CIS	0.034	0.058	−0.080	0.082	−0.036	0.001; 0.013
γ-CD	δ	5.120	3.663	3.944	3.600	3.867	3.879
	CIS	0.029	0.001	−0.051	0.005	−0.114	−0.016; −0.059

Table 3. ^{1}H NMR Chemical shifts (δ, ppm), CIS (ppm) and temperature coefficients ($d\delta/dT$, ppb/°C) for OH of β-CD and γ-CD at −10 °C

	OH-2			OH-3			OH-6		
	δ	CIS	$d\delta/dT$	δ	CIS	$d\delta/dT$	δ	CIS	$d\delta/dT$
β-CD	6.39	−0.22	−4.2	6.71	−0.47	−0	6.06	−0.12	−10.2
γ-CD	6.43	−0.07	−6.4	6.71	−0.17	−6.4	6.00	−0.03	−11.4

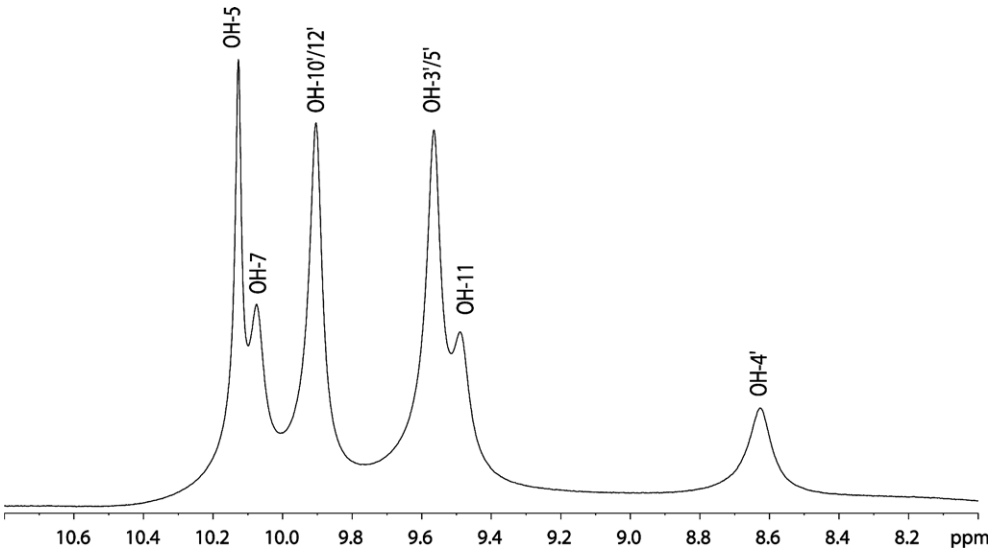

Figure 3. Part of the 1D ^{1}H NMR spectrum of EGCG showing the hydroxy proton region (85% H_2O/15% $(CD_3)_2CO$, −15 °C, pH 4.4).

Table 4. ^{1}H NMR Chemical shifts (δ, ppm), CIS (ppm) and temperature coefficients ($d\delta/dT$, ppb/°C) for OHs of EGCG at −10 °C

	OH-5	OH-7	OH-10′/12′H	OH-3′/5′	OH-11′	OH-4′
δ	10.08	10.03	9.88	9.57	9.57	8.6
$d\delta/dT$	−9.1	−9.1	−4.9	−8.2	−8.2	[a]
CIS (β-CD)	−0.47	−0.14	−0.10	−0.09	[a]	0.12
$d\delta/dT$ (β-CD)	−2.3	[a]	[a]	−5.9	[a]	−12.2
CIS (γ-CD)	−0.31	−0.40	−0.05	0.02	[a]	0.05
$d\delta/dT$ (γ-CD)	−3.0	−1.8	−13.7	−8.1	[a]	−11.4

[a] Could not be determined due to broadening of resonance lines or spectral overlap.

(Table 4). A comparison of the chemical shifts of these hydroxy proton signals with those reported for flavones and flavonol[22] indicated that the hydroxy protons of EGCG were not involved in a strong intramolecular hydrogen bond interaction. In the example of kaempferol, an intramolecular hydrogen bond interaction resulted in a downfield shift of the OH-5 proton signal to δ 12.5 ppm.[22]

2.2.4.2. Hydroxy protons of β-CD in the complex. The NMR spectra of hydroxy protons of β-CD showed drastic changes upon addition of EGCG with line broadening and upfield shifts of the OH-3 and OH-2 signals by 0.47 and 0.22 ppm, respectively (Table 3). Upfield shifts of hydroxy proton resonances have previously been attributed to reduced hydration.[21,23] The absence of chemical shift changes observed for OH-3 in the temperature range −10 to +9 °C and the low temperature coefficient (−4.2 ppb/°C) of OH-2 (Table 3) indicated also less accessibility to water upon complexation. In β-CD alone, temperature coefficient values of −7.5 and −8.7 ppb/°C were measured for OH-2 and OH-3, respectively.[21] Thus, upon inclusion of EGCG, water is excluded from the CD cavity and the hydration of OH-2 and OH-3 is reduced. The differences in the complexation induced shift (CIS) and temperature coefficients of the two hydroxy protons are due to different positions on the β-CD cavity rim, OH-2 being more exposed to the surrounding water. The smaller chemical shift change and higher temperature coefficient of OH-6 (Table 3) indicate more contact with water. No intermolecular ROE or chemical exchange interaction could be observed between the hydroxy protons of β-CD and the hydroxy protons of EGCG because the optimum pH for the observation of hydroxy protons in the two compounds was too different (5.8 for β-CD and 4.4 for EGCG). Thus, from the data available, it is not possible to conclude whether the intramolecular OH-3–OH-2 hydrogen bond existing in β-CD is replaced by intermolecular hydrogen bonding with the OH protons of ring B and C of EGCG. However, if these two OH protons had only reduced hydration, their resonance lines should be sharp.[23] The observed broadening is tentatively assigned to the existence of transient intermolecular hydrogen bonds. Indeed, modelling using MM2 calculations and ROE data showed that the secondary hydroxy groups of β-CD have the proper distance and orientation to form hydrogen

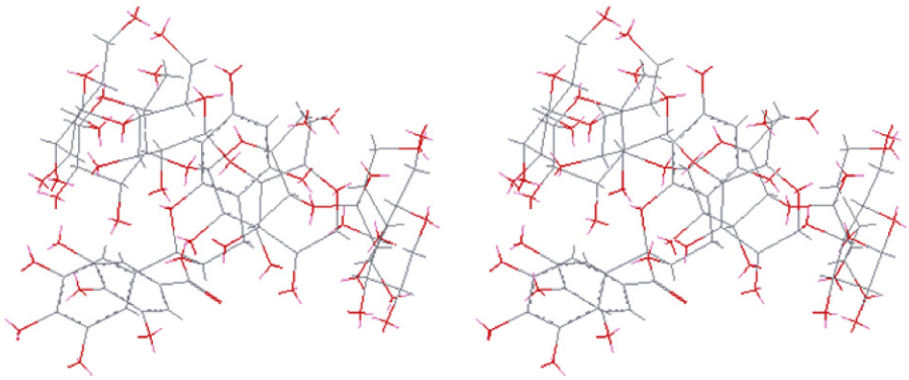

Figure 4. Stereoview of a possible structure of the inclusion complex formed between β-CD and EGCG. The figure was made with the program Chemdraw 3D Ultra. The structure was derived from the data obtained from the hydroxy protons and ROEs.

bonding with the carbonyl group of the gallate as well as with OH-3′/5′ and OH-10′/12′ of rings B and B′ (Fig. 4).

2.2.4.3. Hydroxy protons of EGCG in the complex. In the 1:1 complex, the resonances of the external hydroxy protons in the B and B′ rings showed no major changes in chemical shifts (Table 4) but important line broadening. Similarly, the OH-7 signal in ring A was broader upon complexation but no significant chemical shift change was observed. The OH-5 appeared on the other hand as a sharp signal shielded by 0.47 ppm and this had intramolecular ROE to H-6 and intermolecular ROE to H-5 of β-CD. Together with a low temperature coefficient of −2.3 ppb/°C (−9.1 ppb/°C in EGCG alone), these data indicate that the OH-5 hydroxy proton of EGCG is located inside the CD cavity.

2.3. Complex formation between EGCG and γ-CD

The amount of γ-CD coupled to silica gel was determined using the same procedure as for β-CD, and a coupling amount of 5 μmol per gram gel was obtained. As with β-CD bonded on silica beads, for samples in D_2O, H-6 and H-8 of EGCG had completely exchanged with deuterium during the time necessary to form a complex with γ-CD (Fig. 5c). This exchange process could be avoided by using H_2O solution instead of D_2O as was done for the complex with β-CD (vide infra).

In the presence of γ-CD, the proton signals of EGCG did not broaden significantly at room temperature (Fig. 5) indicating less restricted motion than in the presence of β-CD. Significant shifts for the signals of H-3 and H-5 of γ-CD were however observed. In β-CD, H-3 was the most shielded proton whereas in γ-CD, H-5 was con-

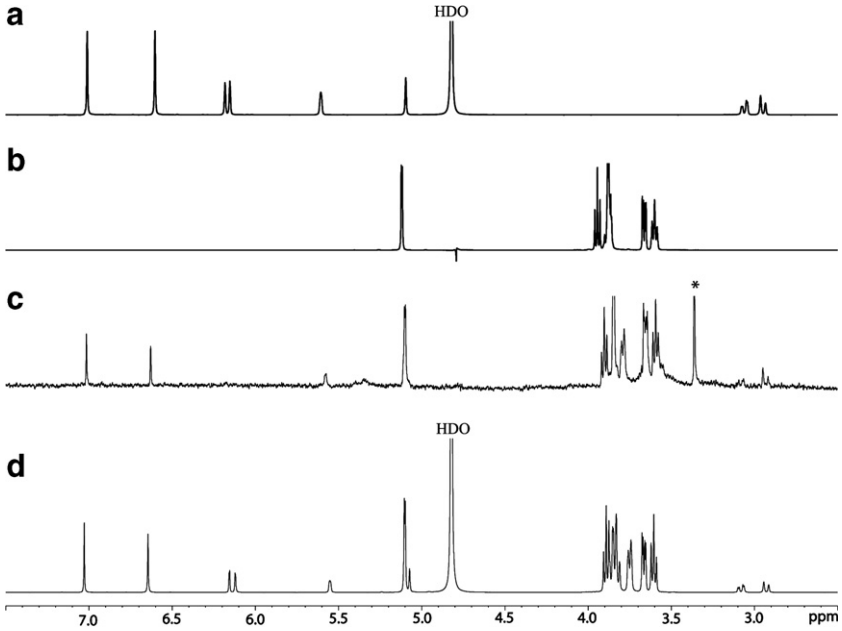

Figure 5. 1D ^1H NMR Spectra (D_2O, 25 °C) of (a) EGCG, (b) γ-CD, (c) γ-CD gel/EGCG (1:1) in a 4 mm HR-MAS probe and (d) γ-CD/EGCG (1:1) in a 5 mm HR probe. During the time necessary for equilibrium, the H-6 and H-8 protons have exchanged with deuterium. ∗: Spacer.

siderably more shielded than H-3 (Table 2). In the wider γ-CD cavity, the guest can penetrate more deeply inside the cavity resulting in that the narrow side protons, H-5, are closer to EGCG and thus more affected. As with β-CD, H-2, H-3, H-4α, H-6 and H-8 of EGCG were shielded upon addition of γ-CD, while the other protons were deshielded. The magnitude of the shifts was however much smaller with γ-CD probably because of the larger distances between the guest and the host molecule. Structural information on the intermolecular interactions was obtained from 2D ROESY NMR spectra. Comparison with the ROESY spectra of the β-CD/EGCG complex showed that the relative intensities of the intermolecular ROEs were smaller. H-9′/13′ and H-2′/6′ of EGCG had ROE to H-3 of γ-CD. Interestingly, no intermolecular ROE was found for H-8 of EGCG, while H-6 only showed medium ROE to H-5 and a weak ROE to H-3 of γ-CD. Both H-4α and H-4β of EGCG showed ROE to H-5 of γ-CD. H-2 of EGCG had ROEs to both H-5 and H-3 of γ-CD, the ROE to H-5 being stronger. H-3 of EGCG also had ROEs, albeit weaker to both H-3 and H-5 of γ-CD, the NOE to H-3 being stronger. These data suggest that EGCG penetrates deeper into the γ-CD cavity than into that of the β-CD, part of the A ring lying outside of the narrow rim of the CD cavity. It also indicates that while EGCG fits tightly with β-CD, it fits more loosely with γ-CD.

Upon inclusion of EGCG, no significant broadening of the hydroxy proton resonances of EGCG or γ-CD was observed. In γ-CD, OH-2 and OH-3 were shielded to a much smaller extent than that in β-CD (−0.07 and −0.17 ppm, respectively) and had a higher temperature coefficient (−6.4 ppb/°C) similar to those of γ-CD alone.[21] Thus, these protons are only slightly less hydrated than in γ-CD alone confirming that EGCG fits more loosely with the larger γ-CD than with β-CD. The OH-5 and OH-7 of EGCG were shielded by −0.31 and −0.41 ppm, respectively, in the 1:1 complex. Since both hydroxy protons also had a low temperature coefficient, it can be concluded that the hydration of these protons is strongly reduced due to their inclusion in the hydrophobic CD cavity. OH-5 has intermolecular ROE to both H-3 and H-5 of γ-CD as well as intramolecular ROE to H-4 of EGCG, while OH-7 has intramolecular ROE to H-8 but no intermolecular ROE. The other EGCG hydroxy proton signals had chemical shifts and temperature coefficients of the same order of magnitude as those for EGCG alone indicating that they are not strongly involved in the interaction with γ-CD.

3. Conclusion

This work has shown that HR-MAS NMR can be used to determine the amount of cyclodextrins coupled to silica beads and to study the structure of the inclusion complexes formed between the CDs and EGCG directly on the stationary phase. The investigation can be done under conditions similar to those used for chromatographic separations.

Nonspecific interactions were not observed, and the structures of the inclusion complexes with free CDs and CDs bonded to the silica beads were very similar indicating that retention occurs exclusively due to interaction with CD. With both β- and γ-CD, the structure of the complex involves penetration of the most hydrophobic rings. EGCG was however found to bind more tightly to β-CD than to γ-CD, and to have the orientation and distances of CO and hydroxy groups to form hydrogen bonding with the hydroxy groups of β-CD.

4. Experimental

The β-CD bonded silica gel ('CYCLOBOND I 2000') and γ-CD bonded silica gel ('CYCLOBOND II') were from ASTEC.

All NMR spectra were recorded on a Bruker DRX 600 spectrometer using a 5-mm $^{1}H/^{13}C/^{15}N/^{31}P$ inverse detection probe and a 4-mm $^{1}H/^{13}C$ HR-MAS SB BL4 probe, both probes equipped with *z*-gradient.

Prior to analysis with HR-MAS NMR, the gels, typically 2 mg dry weight, were submerged in D_2O for 1 h, and carefully inserted into the magic angle-spinning rotor (zirconia, 4 mm outer diameter, spherical sample volume, Bruker, Karlsruhe, Germany). During the experiments, the samples were spun at 3 kHz at the magic angle (54.7°).

For samples in D_2O, sodium 3-trimethylsilyl[2,2,3,3-d_4]propionate (TSP-d_4) was added as a reference (δ_H 0.00 ppm). The amount of β- and γ-CD bonded to the silica beads was quantified by weighting a precise amount of gel (~2 mg in each experiment) and adding a known quantity of TSP-d_4. The 1D proton NMR spectra were recorded with a repetition delay of 10 s and a pulse angle of 30°. The regions for anomeric and other ring protons of β-CD were integrated relative to that of the TSP-d_4 signal. The experiment was repeated three times for each cyclodextrin.

The samples of CD complexes were prepared to have 5 mM concentrations and a 1:1 molar ratio for both host and guest molecule. NMR spectra of single compounds of α-, β- and γ-CD and of EGCG were recorded alone at the same concentrations as those used to study the complexes.

4.1. Experiments in 15% $(CD_3)_2CO$/85% H_2O

The compounds were dissolved in the solvent mixture to a final concentration of 5 mM to avoid self-aggregation of EGCG. The pH was adjusted in order to obtain sharp resonances for the hydroxy protons using minute

amount of concentrated HCl or NaOH solutions. The optimum pH for the observation of OH of CDs was 5.8 while the optimum pH for the observation of OH of EGCG was 4.4. To minimize the absorption of impurities from the glassware, the NMR tubes were soaked for 24 h in 50 mM sodium phosphate buffer pH 7.[24] One- and two-dimensional ^{1}H NMR spectra were acquired using the WATERGATE pulse sequence[25] for water suppression and the calibration done by setting the residual acetone-d_5 signal to δ_H 2.204 ppm. The 2D DQF-COSY, TOCSY, and ROESY spectra were acquired with standard pulse sequences from the BRUKER library.

4.2. MM2 Calculations

Chem3D Ultra version 9.0.1 for PC was used. The coupling constants of protons in EGCG were the same as the one reported in Ref. 18 and the starting structure for EGCG was taken from Ref. 18. Minimization was performed with the 'MM2' force field. The default convergence criterion was used (rms [root mean square] force 0.1 kcal mol^{-1} A^{-1}).

Acknowledgements

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References

1. Yokozawa, T.; Cho, E. J.; Hara, Y.; Kitani, K. *J. Agric. Food Chem.* **2000**, *48*, 5068–5073.
2. Kawase, M.; Wang, R.; Shiomi, T.; Saijo, R.; Yagi, K. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2218–2220.
3. Mabe, K.; Yamada, M.; Oguni, I.; Takahashi, T. *Antimicrob. Agents Chemother.* **1999**, *43*, 1788–1791.
4. Yee, Y.-K.; Koo, M. W.-L. *Aliment. Pharmacol. Ther.* **2000**, *14*, 635–638.
5. Yang, C. S.; Maliakal, P.; Meng, X. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 25–54.
6. Yamaguchi, K.; Honda, M.; Ikigai, H.; Hara, Y.; Shimamura, T. *Antivir. Res.* **2002**, *53*, 19–34.
7. IlKim, J.; Hong, S. B.; Row, K. H. *J. Chromathogr. A* **2002**, *949*, 275–280.
8. Baumann, D.; Adler, S.; Hamburger, M. *J. Nat. Prod.* **2001**, *64*, 353–355.
9. Kang, J. H.; Chung, S. T.; Go, J. H.; Row, K. H. *J. Liq. Chrom. Rel. Technol.* **2000**, *23*, 2739–2749.
10. Cao, X.; Ito, Y. *J. Liq. Chrom. Rel. Technol.* **2004**, *27*, 145–152.
11. Schneider, H.-J.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755–1786.
12. Liu, L.; Guo, Q. X. *J. Inclusion Phenom. Macrocycl. Chem.* **2002**, *42*, 1–14.
13. Crini, G.; Bourdonneau, M.; Martel, B.; Piotto, M.; Morcellet, M.; Richert, T.; Vebrel, J.; Torri, G.; Morin, N. *J. Appl. Polym. Sci.* **2000**, *75*, 1288–1295.
14. Händel, H.; Gesele, E.; Gottschall, K.; Albert, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 439–442.
15. Hellriegel, C.; Skogsberg, U.; Albert, K.; Lämmerhofer, M.; Maier, N. M.; Lindner, W. *J. Am. Chem. Soc.* **2004**, *126*, 3809–3816.
16. Xu, J.; Sandström, C.; Janson, J. C.; Tan, T. *Chromatographia* **2006**, *64*, 7–11.
17. Xu, J.; Zhang, G.; Tan, T.; Janson, J. C. *J. Chromatogr. B* **2005**, *824*, 323–326.
18. Ishizu, T.; Hirata, C.; Yamamoto, H.; Harano, K. *Magn. Reson. Chem.* **2006**, *44*, 776–783.
19. Charlton, A. J.; Baxter, N. J.; Khan, M. L.; Moir, A. J. G.; Haslam, E.; Davies, A. P.; Williamson, M. P. *J. Agric. Food. Chem.* **2002**, *50*, 1593–1601.
20. Hayashi, N.; Ujihara, T.; Kohata, K. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2512–2518.
21. Bekiroglu, S.; Kenne, L.; Sandström, C. *J. Org. Chem.* **2003**, *68*, 1671–1678.
22. Exarchou, V.; Troganis, A.; Gerothanassis, I. P.; Tsimidou, M.; Boskou, D. *Tetrahedron* **2002**, *58*, 7423–7429.
23. Bendeby, B.; Kenne, L.; Sandström, C. *J. Inclusion Phenom. Macrocycl. Chem.* **2004**, *50*, 173–181.
24. Adams, B.; Lerner, L. E. *Magn. Reson. Chem.* **1994**, *32*, 225–230.
25. Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661–665.