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A Combined NMR, Computational, and HPLC Study of the Inclusion of Aromatic and Fluoroaromatic Compounds in Cyclodextrins as a Model for Studying Carbohydrate–Aromatic Interactions

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A combined NMR, computational, and HPLC study of the inclusion of aromatic and fluoroaromatic compounds in cyclodextrins has been carried out with the aim of studying this process as a model for carbohydrate···aromatic interactions. NMR experiments showed that although β-CD formed an inclusion complex with benzene, no stable inclusion adduct was formed with hexafluorobenzene. MM3* calculations confirmed these data and, when extended to naphthalene, anthracene, phenanthrene, and their partially and fully fluorinated analogues, showed that β-CD formed inclusion adducts only with the hydrocarbons, whereas the partially fluorinated derivatives were shown to enter the cavity only with their non-fluorinated part, and the perfluoro compounds were not included. HPLC experiments, carried out by eluting these compounds through CD-modified HPLC columns, also confirmed these results, at least with the mono- and bicyclic arenes. Indeed, the elution of these derivatives showed that the efficiency of the inclusion, as determined by comparing HPLC retention times, decreased on passing from hydrocarbons to partially fluorinated compounds, and reached a minimum with perfluorinated derivatives. In contrast with this general trend, 1,2,3,4-tetrafluoroanthracene had longer retentions times on β- and γ-CD-modified HPLC columns than anthracene, possibly for solubility reasons. As a whole, these results were interpreted in terms of carbohydrate···aromatic interactions between the C5–H5 bonds on the hydrophobic faces of the sugars and the π electrons of the aromatic partner. The interactions seem to have an important dispersive component.

Introduction

Many biological recognition processes are mediated by interactions between proteins and the carbohydrates present on the external surface of cell membranes.[1] The selectivity and specificity of the carbohydrate···protein interaction depend on a variety of factors. In the last few years it has become clear that, apart from hydrogen-bonding and solvation effects, the presence of aromatic rings at the binding sites of lectins is essential for recognizing neutral sugars. In particular, both the structure and conformation of the carbohydrate,[2] as well as the nature and orientation of the aromatic rings, are of paramount importance.[3] Hydrophobic faces of saccharides, featuring several non-polar C–H groups, indeed interact with the aromatic residues of protein side-chains.[4] This fact has been proved experimentally (e.g., by measuring affinities, but also by NMR spectroscopy and X-ray diffraction) and theoretical models have demonstrated[5] that CH···π interactions play an important role in stabilizing carbohydrate–protein complexes. This interaction has also been shown to occur in the gas phase.[6]

The chemical nature of the aromatic ring seems to play a key role in the binding process.[7] Despite the fact that, for certain hevein domains interacting with chitooligosaccharides, there are no major changes in the binding enthalpy when changing the key interacting residue from tyrosine to tryptophan,[8] in the related AcAMP2 peptide series, significant differences have been found when changing one of the key residues from phenylalanine to tryptophan to naphthylalanine.[9] The binding affinity systematically increased with the size and electronic richness of the aromatic moiety. Moreover, the binding affinity suffered a decrease of about one order of magnitude when phenylalanine was changed to 4-fluorophenylalanine.[9]

Understanding the carbohydrate···aromatic interaction phenomenon might be of crucial importance for the design of glycomimetics that may act as potential drugs or inhibitors in carbohydrate-mediated pathogenic processes. Thus,

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different authors have verified that these interactions do actually occur in simpler systems, not only in protein receptors. For instance, carbohydrate···arene interactions have also been shown to direct the conformational equilibrium of glycophanes,[10] oligosaccharide mimics,[11] DNA-based glyco-oligoamide moieties in water solution,[12] and aromatic-protected sugar derivatives in less polar solvents.[13] It has also been reported by our group that upon the addition of a given aromatic molecule such as phenol to an NMR tube containing an aqueous solution of a simple monosaccharide such as β-methyl galactoside, specific upfield shifts of the ^{1}H NMR resonances of the monosaccharide were observed.[14] This behavior has been taken as a direct demonstration of the existence of sugar–aromatic CH···π interactions,[15] which has also been claimed to bias the conformational behavior of certain glycopeptides in water solution.[16]

The presence of three C–H vectors pointing towards the same spatial orientation seems to be a key factor in defining the stabilization of the intermolecular carbohydrate···aromatic interaction. Nevertheless, preliminary results obtained from a comparison of the NMR spectroscopic data of some of these molecules in water compared with those measured in nonaqueous solvents, such as CD_3OD or DMSO,[17] seem to indicate a weakening of the observed effects in the NMR spectra, and possibly suggest a role for hydrophobic packing in defining the interaction.

Cyclodextrins (CD) are paradigmatic molecules for mimicking molecular interactions that occur in Nature.[18] They are able to form inclusion compounds with a variety of organic molecules, with hydrophobic and van der Waals effects occurring within the cyclodextrin cavity, which acts as driving forces for the recognition process.[19]

On this basis we have decided to use these well-characterized simple molecules as targets for the recognition of a variety of aromatic and fluoroaromatic compounds having different chemical natures, sizes, and electronic distributions (see Figures 3, 4, 5, 6, 7, and 8 for the structures of the aromatic compounds). A combined multidisciplinary approach based on NMR,[20] molecular mechanics calculations,[21] and HPLC[22] has been chosen to explore these interactions.

Results and Discussion

NMR Spectroscopy

Chemical shift values of molecules in solution are very sensitive to environmental changes (e.g., solvent, temperature, neighboring molecules). Thus, the observation of chemical shift variations is crucial for the identification of protein···ligand interactions,[23] the determination of binding constants,[24] and the elucidation of protein secondary structures.[25]

Thus, as a first step, 500-MHz ^{1}H-NMR titrations of β-CD with different compounds were performed by using standard methodology. Figure 1 shows the variations in chemical shifts observed in the ^{1}H NMR spectra of β-CD in water (in the presence of 10 μL of methanol due to solubility problems, 2% volume in the NMR tube) upon titration with benzene.

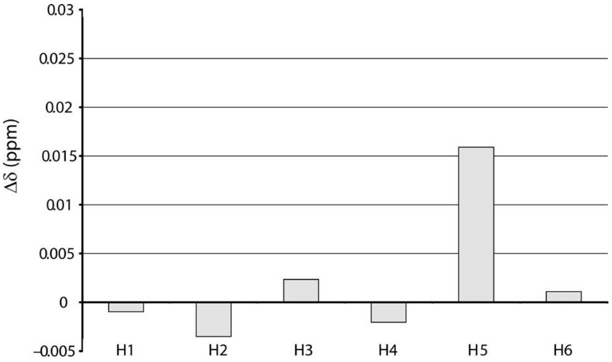

Figure 1. Variations of the chemical shifts of the β-CD protons upon addition of increasing amounts of benzene to the NMR tube with a constant concentration of β-CD. Upfield shifts are observed for 3-H and, particularly, for 5-H. In contrast, the chemical shifts of the benzene protons upon addition of increasing amounts of β-CD to the NMR tube with a constant concentration of benzene showed the opposite tendency compared with 3-H and 5-H of the cyclodextrin. The data were recorded at 500 MHz and 298 K in D_2O with 2% of methanol for solubilizing benzene. Molar ratios of 90:1 were reached.

As can be seen, a remarkable upfield shifting effect on the chemical shift value of 5-H of β-CD is revealed. The chemical shifts of the other protons are much smaller. This fact is a clear indication of the inclusion of the aromatic moiety within the CD cavity. The Job plot (Figure 2) indicates the presence of a 1:1 molecular complex, and the association constant was estimated to be around 350 M^{-1} at room temperature. The NMR signals of benzene were also strongly affected (downfield shifted) when increasing amounts of β-CD were added to a solution of benzene in water (in the presence of 10 μL of methanol to avoid solubility problems, 2% volume in the NMR tube).

The comparison with hexafluorobenzene was then carried out under the same experimental conditions. In this case, the ^{1}H NMR spectrum did not show any resonance shift of the CD protons, which indicates that the fluorinated analogue does not interact with β-CD. The same happened to the ^{19}F signals of the aromatic compound upon addition of β-CD. The binding affinity, thus, should be below 1 M^{-1} at best.

Larger aromatic moieties were then studied. For naphthalene, as well as for its tetra- and octafluoro analogues, deuteriated DMSO was used as the solvent in the NMR experiments due to solubility problems. The addition of water, even in small amounts, precluded these experiments. Therefore, no binding affinity in water could be estimated. By using DMSO, none of the chemical shifts of any molecule (CD or aromatic moieties) were perturbed. Thus, in these cases, the binding affinity should also be below 1 M^{-1}. It seems that water is required to be able to detect the interaction of CD with these aromatic moieties.

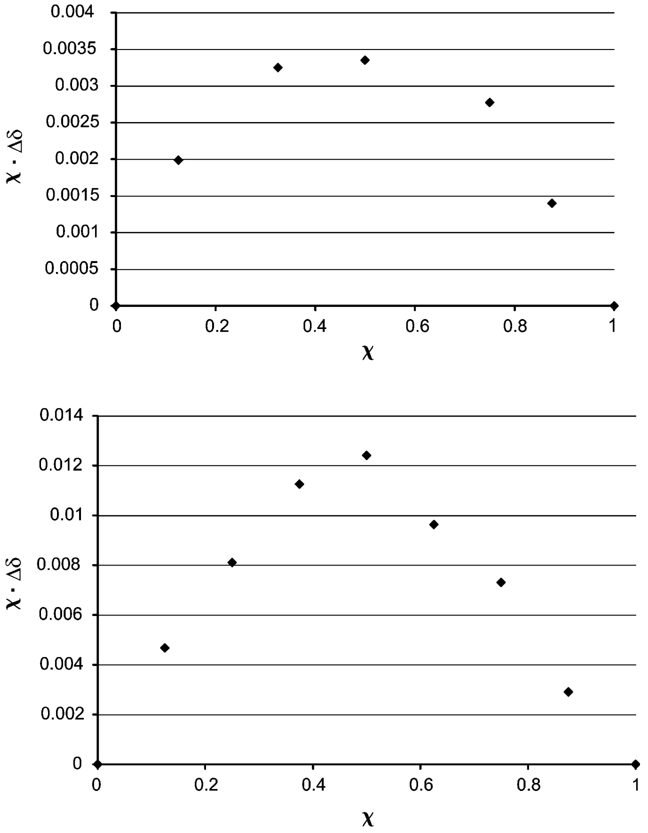

Figure 2. Job plots for the direct and inverse titrations of β-CD protons with benzene, as deduced by NMR experiments. Top: variation of the β-CD protons. Bottom: variation of the benzene protons. Only absolute values are given; the variations for the cyclodextrin and benzene protons follow opposite trends.

A 3D Model: Molecular Mechanics and Dynamics Calculations

As additional support to verify the existence (or absence) of the complexes, molecular mechanics calculations were performed on the different CD···aromatic systems.[21] We decided to resort to molecular mechanics and dynamics calculations due to the speed of the calculations and to the fact that dispersion of electronic density and van der Waals forces seem to be the key interactions in the process. The corresponding terms should be properly parametrized in the force fields currently used to deal with organic molecules, for example, MM3*. Thus, different calculations on the interaction of β-CD with benzene and hexafluorobenzene, naphthalene, 1,2,3,4-tetrafluoronaphthalene (two possible orientations within the CD cavity), and octafluoronaphthalene, anthracene and 1,2,3,4-tetrafluoroanthracene (two possible orientations within the CD cavity), and phenanthrene and 1,2,3,4-tetrafluorophenanthrene (two possible orientations within the CD cavity) were performed.

When the calculations, using the explicit solvent model for water (GB/SA),[26] were performed for the inclusion of benzene within the cavity, the included ring remained at that position, showing oscillations around the central position. MD simulations were performed at different temperatures to show the stability of the complex (Figure 3).

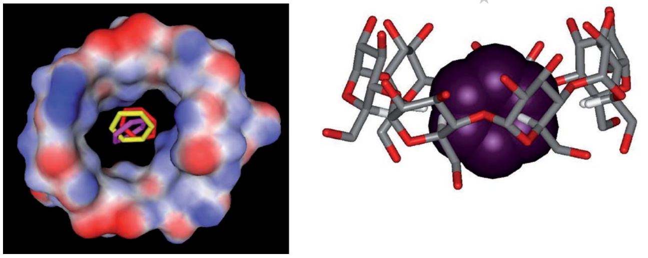

Figure 3. The interaction of β-CD with benzene according to MM3* calculations. On the left-hand side, snapshots of three frames from the AMBER-based MD simulation carried out in explicit water at 300 K. On the right-hand side is the final point of a GB/SA MM3*-based MD simulation carried out at 200 K and subsequently minimized. The distance from the center of the benzene moiety to the center of the plane defined by O-2 and O-3 is 3.22 Å. The benzene fits well within the cavity and the different C5–H5 bonds of β-CD point towards the aromatic molecule.

It was observed that between 100 and 300 K the trajectories were stable and showed the included molecule. A view of the orientation of the ring within the cavity, with close contacts between the C5–H5 bonds of the cyclodextrin and the aromatic ring, is shown in Figure 3. In this case the simulation was conducted at 200 K.

MD simulations using explicit solvent (TPI3P water) were also performed at 300 K and 1 atm. The system was stable for the 2 ns simulation and benzene was maintained included within the cavity for the whole run, which resembles the situation described above for the GB/SA simulation.

Next, the same calculations were conducted for hexafluorobenzene by using both the GB/SA solvent model and explicit water. The minimization carried out by using molecular mechanics calculations led to a complex the energy of which was much higher (above 10 kcal/mol) than that of the previous CD···benzene complex. Both the explicit water (Figure 4) and the GB/SA-based simulations led to the dissociation of the complex with the aromatic C_6F_6 ring well outside the cavity. Even at 100 K (GB/SA), and starting from the inclusion compound, the complex dissociated immediately (Figure 4).

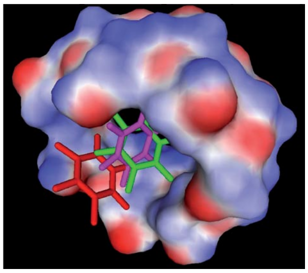

Figure 4. The interaction of β-CD with hexafluorobenzene. Although during MM3* minimization the hexafluorobenzene is kept within the cavity, the complex is immediately dissociated during the MD simulation, irrespective of whether the simulation is carried out with the explicit water or the GB/SA solvent model, and even at a very low simulation temperature. The figure shows the C_6F_6 ring outside of the CD ring.

Then the complexation of higher aromatics was explored. The same MM/MD protocol was applied to the complex formed by β-CD and naphthalene. In this case, different calculations were carried out, either by using the continuum solvent model for water (GB/SA)[26] or by employing a bulk dielectric constant of 47.5 to mimic DMSO as the solvent (as employed in the NMR experiments). Depending on the chemical nature of the aromatic ring, different starting geometries were employed with different sides of the aromatic embedded within the cavity (with hydrogen or fluorine atoms).

The geometries obtained from the two computational models were quite different (as observed in Figure 5). For the calculations in water, the naphthalene ring is completely embedded within the cavity, which parallels the situation described above for benzene. In the case of the simulation that mimics DMSO conditions, the interactions between the aromatic moiety and the CD cavity are very superficial and do not lead to major intermolecular contacts. Moreover, the toroidal shape of the CD molecule is significantly distorted upon binding to the aromatic molecule.

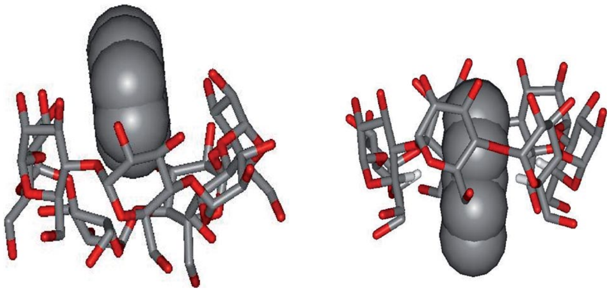

Figure 5. Models of the putative inclusion of a naphthalene molecule in β-CD. On the left-hand side, the model depicts the structure calculated by using the GB/SA continuum solvent model for water. The naphthalene ring is well embedded within the cavity, especially one of the two rings. On the right-hand side is the 3D model obtained when using a dielectric constant of 47.5, which mimics the behavior in DMSO. Very weak interactions are observed, but neither major contacts nor inclusion within the cavity. A clear distortion of the toroidal shape of β-CD is evidenced to allow these intermolecular contacts.

This situation is even more evident for the putative complexes of β-CD with the tetra- and octafluorinated naphthalene analogues (Figure 6). For 1,2,3,4-fluoronaphthalene in water, the global minimum shows that only the non-fluorinated ring is partially embedded (but not the fluorinated ring) within the cavity, making contacts with the cyclodextrin molecule. When the calculations were started with the fluorinated moiety inside the cavity, dissociation took place. Moreover, when the calculations were performed by using a dielectric constant of 47.5, mimicking the behavior in DMSO, very minor interactions were observed, although some distortion of the toroidal shape of the cyclodextrin was evidenced. Also, this complex was not stable under these conditions and dissociated after a few picoseconds in the MD simulation, even at 100 K. When the simulations were performed with octafluoronaphthalene, the aromatic molecule left the cavity immediately, paralleling the behavior of hexafluorobenzene depicted in Figure 4.

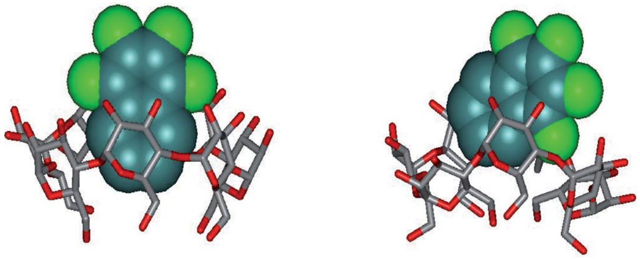

Figure 6. Models of the putative inclusion of 1,2,3,4-tetrafluoronaphthalene in β-CD. On the left-hand side, the model depicts the best calculated structure using the GB/SA continuum solvent model for water. Two possible orientations, with hydrogen or fluorine atoms pointing towards the cavity, were tried as the initial structures. The non-fluorinated ring is partially embedded within the cavity, making some contacts with the cyclodextrin molecule. On the right-hand side is the 3D model obtained when using a dielectric constant of 47.5, which mimics the behavior in DMSO. Basically, no weak interactions are observed, although some distortion of the toroidal shape of the cyclodextrin is evidenced. This complex is not stable and dissociates during a 1 ns MD simulation, even at 100 K. When the simulations were performed with the octafluoronaphthalene analogue, the aromatic molecule left the cavity immediately, resembling the behavior of hexafluorobenzene, as depicted in Figure 5.

MM/MD calculations for the interaction of tricyclic aromatic molecules were also performed. Thus, anthracene, phenanthrene, and their 1,2,3,4-tetrafluoro derivatives were also studied (Figures 7 and 8).

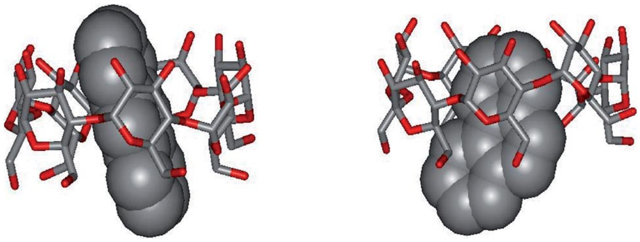

Figure 7. Models of the putative inclusion of anthracene (left) and phenanthrene (right) in β-CD using the continuum solvent model. In both cases, the structures were calculated by using the GB/SA continuum solvent model for water with the aromatic compounds well embedded within the cavity, making key contacts with the cyclodextrin molecule. This is especially the case for anthracene.

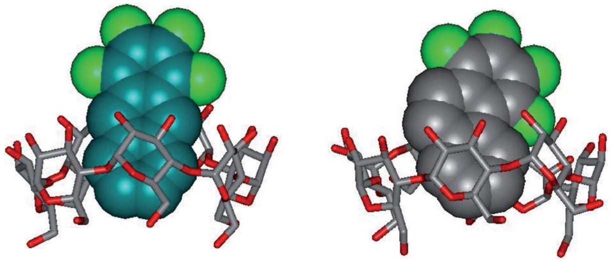

Figure 8. Models of the putative inclusion of 1,2,3,4-tetrafluoroanthracene (left) and 1,2,3,4-tetrafluorophenanthrene (right) in β-CD. Two possible orientations, with the hydrogen or fluorine atoms pointing towards the cavity, were tried as initial structures. The depicted structures are those with better energy values. In both cases, the structures were calculated by using the GB/SA continuum solvent model for water, and show partial contacts of the non-fluorinated rings with the CD cavity. The fluorinated rings are in each case well outside the CD cavity.

In both cases, the MM and MD simulations were performed by using the GB/SA continuum solvent model for water. As for 1,2,3,4-tetrafluoronaphthalene in water solution, the global minimum shows that only non-fluorinated rings were partially embedded within the cavity, making contacts with the cyclodextrin molecule. Again, only when the starting geometry presented the hydrogen-containing moiety within CD did the complexes remain stable. In those cases in which complexes were formed, the fluorinated rings are located well outside the CD cavity.

Correlation with HPLC Experimental Data

The commercial availability of HPLC columns modified with α-, β-, and γ-cyclodextrins suggested the possibility of studying the elution behavior of aromatic and fluoroaromatic compounds to qualitatively deduce how the CD···aromatic interaction was influenced by the presence of fluorine substituents on the arenes. In similar HPLC investigations of the inclusion of polycyclic aromatic hydrocarbons,[22] it was assumed that a more favorable inclusion should result in longer retention times (R_t). In those studies, the inclusion phenomenon was assumed to essentially depend on purely geometric factors, or, in other words, on how nicely the aromatic molecules fitted within the CD cavity. However, the electronic properties of the aromatic guest, by influencing the dispersion effects involved in the inclusion event, should also affect the R_t. We reasoned that, by comparing aromatic and fluoroaromatic molecules having a homogeneous macroscopic structure (same number of rings and overall molecular shape) and differing only in the presence of H or F atoms, the influence of the geometric component of the recognition process should be minimized and the contribution of electronic factors should be maximized.

Therefore, several of the above-mentioned aromatic and fluoroaromatic compounds (benzene, hexafluorobenzene, naphthalene, 1,2,3,4-tetrafluoronaphthalene, octafluoronaphthalene, anthracene, and 1,2,3,4-tetrafluoroanthracene) were eluted through α-, β, and γ-cyclodextrin-modified HPLC columns. The observed R_t values are reported in Table 1, together with the calculated volumes of the arenes and the R_t values observed when these compounds were eluted through a standard reversed-phase HPLC column (LiChrospher RP-18).

Remarkably, no direct dependence of R_t on fluorine substitution was observed by using the RP-18 column. On the other hand, an inverse relationship between R_t and the fluorine content was observed when all the tested compounds were eluted through the α-cyclodextrin-modified column. In this case, however, the internal cavity of α-cyclodextrin (174 Å^3) can be too narrow to allow an easy inclusion of the aromatic molecules (with a possible exception of the relatively small benzene and hexafluorobenzene molecules) and information of limited relevance can be obtained by comparing these short R_t values.

In contrast, the effect exerted by an increase of fluorine content on the value of R_t of the mono- and bicyclic compounds observed with the β-cyclodextrin-modified column seemed more significant. The observed trend is in full agreement with the predictions made on the basis of the calculations, that is, firmly embedded hydrocarbons and more loosely interacting partially and fully fluorinated derivatives. It is worth mentioning that the size of the cavity of β-cyclodextrin (262 Å^3) seems to be large enough to allow sterically unhindered inclusion of these guest molecules, but not too large to either prevent an effective CD···aromatic contact or to let the included molecule slip too easily out of the cavity. The distance between the CD inner wall and the surface of the aromatic compound appears to be a crucial issue because too long a distance would negatively affect an inclusion phenomenon with a strong dispersive component inversely proportional to the sixth power of the distance between the interacting bodies.

An inverse relationship between R_t and fluorine content was also observed for the compounds of the naphthalene series with the γ-cyclodextrin-modified column. For this CD, the volume of the cavity (427 Å^3) is too large for benzene and hexafluorobenzene complexation, but still suitable for a significant interaction with the bicyclic derivatives. The fact that the R_t observed for naphthalene with the smaller β-cyclodextrin is almost twice that observed with the larger γ-cyclodextrin seems to confirm the importance of the dispersive component in influencing the inclusion.

Table 1. R_t of aromatic and fluoroaromatic compounds on different HPLC columns (sample concentration ca. 10^{-5} M in MeOH).

Compound	Volume [Å^3][a]	R_t [min]			
		α-CD[b,c]	β-CD[b,d]	γ-CD[b,e]	RP-18[f]
Benzene	83.8	3.51	25.31	5.00	–
Hexafluorobenzene	106.1	3.22	4.64	6.12	–
Naphthalene	126.5	4.30	45.63	24.09	5.68
1,2,3,4-Tetrafluoronaphthalene	140.8	3.96	19.88	17.38	8.97
Octafluoronaphthalene	156.4	3.52	7.28	6.86	7.41
Anthracene	167.9	6.70	75.07	33.80	10.66
1,2,3,4-Tetrafluoroanthracene	182.7	6.59	>120[g]	39.13	21.36

[a] Volume calculated for optimized structures (MacroModel 9.1). [b] Eluent: MeOH/water, 25:75; flow rate: 1.0 mL/min; λ: 254 nm. [c] Column: α-Cyclobond; volume of the inner cavity of α-cyclodextrin: 174 Å^3 (ref.[18]). [d] Column: β-Cyclobond; volume of the inner cavity of β-cyclodextrin: 262 Å^3 (ref.[18]). [e] Column: γ-Cyclobond; volume of the internal cavity of γ-cyclodextrin: 427 Å^3 (ref.[18]). [f] Column: LiChrospher RP-18; eluent: MeOH/water, 85:15; flow rate: 0.9 mL/min; λ: 254 nm. [g] No peak was eluted 120 min after sample injection.

Remarkably, the difference in the R_t values observed for tetra- and octafluoronaphthalene with β- and γ-CD is clearly smaller than that observed with naphthalene. This fact also shows that the nature of the aromatic ring (and therefore the character and availability of the aromatic π cloud) indeed mediates the interaction with sugars and that the interaction is much weaker for the fluorinated aromatic compounds.

However, different observations were made for the anthracene derivatives. In this case, the observed effects cannot be solely explained on the basis of CH···π interactions. The R_t values for 1,2,3,4-tetrafluoroanthracene with both β- and γ-cyclodextrin-modified columns are longer than those observed for anthracene. Nevertheless, and based on the MM3*-calculated complexes, it is possible to propose a tentative explanation. The 1,2,3,4-tetrafluoroanthracene molecule enters the cavity with its non-fluorinated terminal ring with the fluorinated segment outside the cavity. Thus, this moiety can easily form additional (polar) C–F···H–O contacts with the hydroxy groups of the cyclodextrin, thus affording a more stable adduct than that with anthracene. Indeed, inspection of the computed 1,2,3,4-tetrafluoroanthracene–CD complex (see Figure 8, left) shows that the fluorine atoms in the 1- and 4-positions are located less than 3 Å from two opposite OH residues. Nevertheless, this interpretation should be considered as merely speculative, and can be easily disputed, because it is generally agreed that aromatic fluorines barely accept hydrogen bonds[27] and the existence of a C–F···H–O interaction in solution has never been unambiguously demonstrated.[28]

On this basis, it also seems possible to interpret the observed R_t values simply by considering that anthracene and 1,2,3,4-tetrafluoroanthracene interact similarly with the CD cavity because the interactions involve non-fluorinated rings and that the lower solubility of the fluorinated aromatic compound in the eluent accounts for its longer R_t. This explanation is in part supported by the much longer R_t observed for 1,2,3,4-tetrafluoroanthracene than for anthracene on the RP-18 column.

Discussion

Very recently, and in an effort to mimic Nature, a synthetic lectin was chemically prepared by combining different molecular fragments that allow hydrogen bonds and stacking interactions to be established simultaneously with the target saccharides.[29] On this basis, the understanding of the relative role of polar[30] and apolar interactions for saccharide recognition is of paramount importance. The NMR and HPLC experimental data agree with the calculations in showing that aromatic hydrocarbons form much stronger complexes with CD than their perfluorinated analogues, and, accordingly, that partially fluorinated polycyclic compounds enter the cavity of CDs with their non-fluorinated rings. These findings are in line with the only two literature reports that describe the inclusion of fluorinated aromatic compounds in CDs. In 1992, Shibakami and Sekiya[31] showed, by X-ray crystallography, that α-CD forms inclusion complexes with 2- and 4-fluorophenol with the fluorine atoms outside the cavity. The authors ascribed these results to the ability of the non-included fluorine atoms to form an intermolecular hydrogen bond with the primary hydroxy group of another CD molecule in the crystal. In 1997, Schneider and co-workers[32] demonstrated that α-CD has a much lower affinity for pentafluorophenol than for phenol (by UV/Vis titration), and that the former enters the cavity only to a very limited extent (by using calculations).

In contrast to the interpretation of F–OH hydrogen bonds as the driving force for fluorine remaining outside the cavity, our results fit within the framework of the modern interpretation of carbohydrate···aromatic interactions.[14,17] This interpretation indicates that the interaction is mediated by the dispersive contact between the π cloud of an electron-rich aromatic ring and the hydrogen atoms of the C–H bonds of the more apolar face of a sugar: The more extended, polarizable, and electron-rich the aromatic residue, the stronger the interaction. This explains not only why electron-rich aromatic hydrocarbons are efficiently included but electron-poor perfluorinated ones are not, but also why molecules like naphthalene and 1,2,3,4-tetrafluoronaphthalene, for which very similar molecular fragments enter the cavity (see Figure 7), are retained to a different extent. Clearly the presence of the electron-withdrawing fluorine atoms in 1,2,3,4-tetrafluoronaphthalene makes, at least in part, the non-fluorinated rings less electron-rich and thus less prone to interact with the CD cavity.

Also worthy of comment are the results of MD calculations that show an included perfluoroaromatic compound such as hexafluorobenzene, even when forcibly embedded in the cavity at a simulated low temperature, spontaneously exits the cavity to locate itself outside the CD. It is generally accepted that the driving force for the inclusion of guest molecules inside CDs is provided by the displacement of "high-energy" water molecules located in a hydrophobic environment by more lipophilic guests.[19] Clearly, benzene and hexafluorobenzene, both poorly soluble in water, should be capable of this displacement. However, although benzene does remain inside the cavity, hexafluorobenzene does not. This seems to indicate that although an included benzene molecule experiences a favorable interaction between its π cloud and the hydrogen atoms of the C5–H5 bonds directed towards the center of the cavity, this is not the case for hexafluorobenzene, the π electrons of which, attracted by the strongly electronegative fluorine atoms, are much less polarizable than those of benzene and thus less available for CH···π interactions.

Conclusions

In conclusion, a combined NMR, computational, and HPLC study of the inclusion of some aromatic and fluoroaromatic compounds in cyclodextrins has been carried out with the aim of studying this process as a model for the carbohydrate···aromatic interaction. NMR experiments showed that although β-CD formed an inclusion complex with benzene, no stable inclusion adduct was formed with hexafluorobenzene. Molecular mechanics and dynamics calculations using explicit and implicit solvent calculations confirmed these NMR spectroscopic data and, when extended to naphthalene, anthracene, phenanthrene, and their partially and fully fluorinated analogues, showed that β-CD formed inclusion adducts only with the hydrocarbons, only the non-fluorinated part of the partially fluorinated derivatives enter the cavity, and that perfluoro compounds were not included. Also HPLC experiments, carried out by eluting the aromatic compounds through CD-modified HPLC columns, confirmed these results, at least with the mono- and bicyclic compounds. Indeed, the elution of these derivatives showed that the efficiency of their inclusion, as determined by comparing HPLC retention times, decreased on passing from hydrocarbons to partially fluorinated compounds to reach a minimum with perfluorinated derivatives. In contrast with this general trend, 1,2,3,4-tetrafluoroanthracene showed longer retention times than anthracene on β- and γ-CD-modified HPLC columns. This observation could be ascribed either to adduct-stabilizing interactions between the fluorine atoms of 1,2,3,4-tetrafluoroanthracene and the hydroxy groups of the cyclodextrin, or, more likely, to the poor solubility of this compound in the eluent.

From a more general perspective, these results as a whole reinforce the notion[14–17] that carbohydrate···aromatic binding involves interactions between C–H bonds on the hydrophobic face of the sugar and the π electrons of the aromatic partner. The presence of electron-withdrawing substituents on the aromatic ring hampers in part, or prevents altogether, the binding event. The observation that the strength of the interaction decreases with increasing distance between the CD inner wall and the aromatic surface, likely suggests that the interaction has a strong dispersive component.

Experimental Section

Materials: Benzene, hexafluorobenzene, naphthalene, octafluoronaphthalene, and anthracene were commercial compounds. They were purified by distillation or crystallization prior to use. 1,2,3,4-Tetrafluoronaphthalene and 1,2,3,4-tetrafluoranthracene were prepared as described previously[33] and purified by crystallization. HPLC was carried out on an Agilent 1100 instrument; Cyclobond and LiChrospher columns were used as purchased.

Theoretical Calculations: Molecular mechanics and dynamics calculations were performed on a personal computer operating under Linux with the MM3* force field,[34] as integrated in the Maestro suite of programs.[35] Coordinates determined previously for the CDs[21] were used as the starting point for the calculations. The aromatic moieties were manually included within the cavity, at different positions, and left free during the minimization process.

For the molecular mechanics calculations, the geometries of the complexes were optimized by using the 200 steepest descent steps followed by 5000 conjugate gradient iterations until the root mean square derivative was less than 0.03 $\text{kJ}\,\text{mol}^{-1}\text{Å}^{-1}$. For the asymmetric fluorinated analogues, the two possible binding modes, with either the fluorinated part inside or outside, were tested.

In the first step, the MD simulations were carried out by using the same MM3* force field and a time step of 1 fs. The equilibration period was 200 ps. After this period, structures were saved every ps for 1–5 ns at three different temperatures, 100, 200, and 300 K. Longer simulations were performed for the non-fluorinated analogues. The GB/SA solvent model for water or a 47.5 dielectric constant value, to mimic DMSO, was employed in the different simulation processes.

For benzene and hexafluorobenzene, additional simulations were carried out in explicit solvent by using TIP3P water. In these cases, each of the different starting geometries was immersed in a periodic water box that extended up to 10 Å away from any solute atom. Periodic boundary conditions were applied and electrostatic interactions were represented by using the smooth particle mesh Ewald method[36] with a grid spacing of 1 Å. Molecular dynamics simulations were carried out at 300 K and 1 atm by using the SANDER module in AMBER 8.[37] SHAKE[38] was applied to all bonds involving hydrogen atoms, and the integration time step was 2 fs. The simulation protocol consisted of a series of progressive energy minimizations followed by a 20 ps heating phase and a 250 ps equilibration period after which a 2 ns unrestricted dynamics simulation was performed.

NMR Spectroscopy: Sample pH values were tested by using a thin electrode (Wilmad) fitted directly in a 5-mm NMR tube. The sample concentrations of the titrated component were between 0.2 and 1.0 mM, the aromatic/CD molar ratio was varied from 0:1 to 90:1 and 1:0 to 1:90. The solutions were not degassed.

All NMR spectra were recorded with a Bruker Avance 500 spectrometer at 298 K (^{1}H frequency of 500 MHz). In every case, the lock signal was adjusted to D_2O. The spectra were processed by using Topspin software (version 1.3, Bruker Biospin Inc.). The resonance of the [D_4]trimethylsilylpropionic acid sodium salt (TSP) was used as external reference.

One-dimensional high-resolution experiments were recorded with 32 K complex data points and 16 scans were collected at a spectral width of 4500 Hz. The original FID was zero-filled to 64K and a Fourier transformation using an exponential window function was applied [exponential multiplication, line broadening (lb) = 1 Hz].

HPLC: Samples were prepared by dissolving a known amount of substance (typically 0.05 mmol) in 100 mL of HPLC grade methanol. If dissolution was not complete, the mixture was heated and the solution was further diluted to reach a concentration of ca. 10^{-5} M. The injected volume was 1 µL. During elution, the column temperature was maintained at 30 °C. Frequent column regeneration was required to preserve column efficiency, avoid peak broadening, and minimize peak asymmetry.

Supporting Information (see footnote on the first page of this article): Details of the MD simulation for the β-cyclodextrin/hexafluorobenzene complex.

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