**FOCUS: NONCOVALENT INTERACTIONS**

**Dication Induced Stabilization of Gas-phase Ternary Beta-Cyclodextrin Inclusion Complexes Observed by Electrospray Mass Spectrometry**

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Electrospray mass spectrometry (ES-MS) is an important tool for characterization of non-covalent binding in the gas phase. In this study, iron (II) has been introduced as a dication to enhance the detection of cyclodextrin (CD) plus aromatic compound complexes in ES-MS. Evidence that a novel ternary complex comprised of one β-CD, one iron (II) and one toluene exists as an inclusion complex has been compiled via ES-MS and ES-MS/MS experiments as well as by a computational approach. This evidence strongly suggests that iron (II) serves to modify the conformation of the β-CD ring, and that toluene inclusion is stabilized by dication interaction with the toluene π-system and by crimping of the β-CD ring leading to stronger van der Waals interactions with toluene. Mg(II), another dication of similar radius, showed similar behavior, while added group one cations (H⁺ and Na⁺) were ineffective at producing observable ions representative of the complex. The ternary β-CD complex with iron (II) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has also been examined. ES-MS and ES-MS/MS experiments suggest that it is the polar portion of 2,4,5-T (i.e., the carboxylic acid moiety) that is favored for inclusion in the β-CD cavity, rather than the non-polar aromatic part. (J Am Soc Mass Spectrom 2003, 14, 449–459) © 2003 American Society for Mass Spectrometry

Cyclodextrins are important cyclic macromolecules that can serve as hosts to form inclusion complexes with compatible guest molecules in the solid [1–3], the liquid [4–6] as well as in the gas [7, 8] phases. Based on the number of 1,4 linked α-D-glucopyranose units in the cyclic oligosaccharide structures, the three most common cyclodextrins can be differentiated as α (6 units), β (7 units) and γ (8 units) forms. These cyclodextrins are characterized by a “torus” shaped structure, with an upper, wider rim consisting of C2, C3 secondary -OH groups and a lower, narrower rim consisting of C6 primary -OH groups. In aqueous solution, the internal cavities of cyclodextrins are hydrophobic, thus making them suitable to host non-polar hydrophobic molecules that fit inside the cavity. This important property of cyclodextrins has been widely applied in pharmaceutical [9], agricultural, food, cosmetic, chemical and many other industries [10, 11].

Compared to the extensive research work on cyclodextrin inclusion complexes in the liquid phase, studies on such host-guest interaction in the gas phase are much more limited. In the gas phase, the influence of the solvent and counter ions (for charged species) on host-guest interaction in cyclodextrin inclusion complexes is removed. An important consequence is that the hydrophobic effect [6], which is a driving force for the formation of cyclodextrin inclusion complexes in aqueous solution, no longer exists in the gas phase where the electrostatic interactions between cyclodextrin and the attaching molecule become dominant. Thus, ionic forms of cyclodextrin complexes observed in the gas phase via mass spectrometry are not necessarily inclusion complexes [12], as the polar part of the attaching molecule might actually be external to the cavity, interacting with hydroxyl groups on the rim of the cyclodextrin ring. Moreover, the presence of one or more charge carriers may also alter the nature and geometry of the interaction between host and guest. Recent studies by Lebrilla and coworkers [8, 13–17] have indicated that in the gas phase, polar moieties of “hydrophilic” molecules such as amino acids could also be inserted into appropriate cyclodextrin cavities to form inclusion complexes. Steric constraints imposed by the chiral cyclodextrin cavities result in a chiral...
selectivity for complex formation. Contrary to previous assumptions that polar moieties always tend to remain external to the cavity, the non-polar aromatic substituents of certain amino acids were proposed to be located outside the cavities. This contention was supported by molecular dynamic simulation results. Gas-phase ion-molecule reactions between protonated cyclodextrin and neutral amines also suggest that amines are included in the cyclodextrin cavity during adduct formation [18]. The above investigations signal that solution-phase cyclodextrin inclusion complexes may change form, or dissociate entirely, upon transfer into the gas phase.

When employing positive ion electrospray (ES) [8, 12–24], ionspray [25–28] or matrix-assisted laser desorption/ionization (MALDI) [29,30] mass spectrometry (MS) to investigate cyclodextrin complexes with attaching neutral molecules, the observed ions usually incorporate a small cation (e.g., H⁺, or alkali metal cations) to make the complex detectable for further studies. In contrast to the group I cations often serving to impart charge, in this work, we introduce Fe(II) and Mg(II) as agents for stabilizing noncovalent complexes of cyclodextrin and neutral aromatic molecules. The structures of Fe(II) cyclodextrin binary complexes and Fe(II) cyclodextrin ternary complexes with polar and non-polar aromatic compounds were investigated via experimental (ES-MS and ES-MS/MS) as well as theoretical approaches.

Experimental

Solution Preparation

Cyclodextrins (α and β) were provided by Cerestar (Hammond, IN). Toluene was purchased from EM Science (Gibbstown, NJ), while iron (II) perchlorate, magnesium (II) perchlorate, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,3-dihydroxynaphthalene came from Aldrich (Milwaukee, WI). All materials were used without further purification. 1 mM cyclodextrin solutions were prepared, and an amount of aromatic compound beyond the saturation limit was added. The mixture was allowed to stand for at least two hours to ensure saturation of the aromatic compound in solution, and to obtain an initial host-guest equilibrium. Afterwards, iron (II) perchlorate solid (or magnesium (II) perchlorate) was added into the solution (to 1 mM) just prior to ES-MS analysis in order to minimize the oxidation of iron (II). After centrifuging the mixture for a few minutes, insoluble substances were removed. The final sample solution contained 1 mM Fe(ClO₄)₂ (or 1 mM Mg(ClO₄)₂), with 1 mM cyclodextrin, and saturated aromatic compound.

Mass spectrometry

All mass spectrometry experiments were performed on a Quattro II triple quadrupole mass spectrometer (Micromass Inc., Manchester, UK) equipped with an electrospray source. The sample solutions were introduced into the electrospray source at 2–4 μL/min. High flow rate nitrogen gas was employed as nebulizing gas as well as drying gas to aid desolvation. The electrospray source temperature was set at 110 °C. Argon collision gas was introduced into the central hexapole collision cell (1.2–1.5 × 10⁻⁴ mbar, external gauge) of the mass spectrometer in collision induced decomposition (CID) experiments. During metastable decomposition experiments, no argon collision gas was introduced and background pressure in the central hexapole collision cell was 1.0–1.2 × 10⁻⁵ mbar.

Molecular modeling

Molecular modeling was performed on a Gateway x86 PC computer using PC SPARTAN Plus version 2.0 [31]; initial structure templates were built by the Molecule Builder integrated into the PC SPARTAN package. The Energy Minimizer (based on the MMFF94 [32] force field) was used to refine the preliminary structure during construction of the structure template. Afterwards, geometry optimizations were performed at the MMFF94 level starting from the structure refined by the Energy Minimizer.

Results and Discussion

Toluene is known to be a highly compatible guest molecule for the β-cyclodextrin (β-CD) cavity. The inclusion complex of toluene and β-CD in solution is well defined and plays an important role in the industrial production of β-CD [33]. In the gas phase, however, it has been reported [12] that the toluene β-CD complex could not be observed via positive mode ES-MS employing either H⁺ or Na⁺ as the attaching cation. In our laboratory, we also tried to use mass spectrometry to observe the complex of toluene and β-CD under various conditions that favor ionic adduct formation, but we were unable to observe any signal above background levels for the ionized complex. It seems that β-CD inclusion complexes with non-polar aromatic guest molecules, such as toluene, held purely by hydrophobic interactions, including van der Waals interactions [33] in aqueous solution, cannot readily be preserved for mass spectrometric detection in the gas phase with a proton or an alkali metal ion acting as the attaching cation. As an alternative approach, we employed the dication Fe(II) that was introduced in salt form. Upon mixing in solution, the binding between β-CD and Fe(II) is clearly quite strong as evidenced by the strong signal corresponding to [β-CD + Fe(II)]²⁺ that appears at m/z 595 in positive mode ES mass spectra (Figure 1).

Preparation of a 1 mM β-CD + 1 mM Fe(ClO₄)₂ saturated toluene (aqueous) solution resulted in the ability to view the ternary complex [β-CD + toluene + Fe(II)]²⁺ complex at m/z 641 (Figure 1b). The control
ES-MS experiment examining only 1 mM β-CD + 1 mM Fe(ClO₄)₂ aqueous solution (no toluene) was also performed and this mass spectrum is shown in Figure 1a.

In comparing Figure 1a and Figure 1b, it is evident that m/z 641 is produced as the result of adding toluene to the 1 mM β-CD and Fe(II) solution.

Even though the abundance of the [β-CD + toluene + Fe(II)]²⁺ ternary complex at m/z 641 is not very high compared to [β-CD + Fe(II)]²⁺ at m/z 595, it is still high enough to perform a tandem mass spectrometry experiment. Figure 2a shows the metastable decomposition spectrum of the m/z 641 precursor; it implies that [β-CD + toluene + Fe(II)]²⁺ is quite stable in the absence of added collision gas. Figure 2b is the CID spectrum of the m/z 641 precursor that was acquired using argon gas at low pressure (0.1–0.2 mTorr) and low collision energy (E_lab = 10 eV). [β-CD + Fe(II)]²⁺ appears as the sole product ion at m/z 595; this ion results from the loss of neutral toluene, thus confirming that m/z 641 indeed represents the ternary complex [β-CD + toluene + Fe(II)]²⁺.

In order to elucidate the structure of the novel

Figure 1. 1 mM β-CD + 1 mM Fe(ClO₄)₂ with and without saturated toluene are examined by ES-MS. (a) ES mass spectrum of 1 mM β-CD + 1 mM Fe(ClO₄)₂ without toluene from m/z 500-1100; the inset shows a blow-up of m/z 110–180 of the same spectrum; (b) ES mass spectrum of 1 mM β-CD + 1 mM Fe(ClO₄)₂ with saturated toluene from m/z 500–1100; inset shows m/z 110–180 of same spectrum. The ion at m/z 595 is the binary complex [β-CD + Fe(II)]²⁺; the ion at m/z 641 is the ternary complex [β-CD + toluene + Fe(II)]²⁺.

Figure 2. Tandem mass spectra of the selected precursor ternary complex [β-CD + toluene + Fe(II)]²⁺ at m/z 641. (a) metastable decomposition of m/z 641; (b) CID of m/z 641.
ternary complex $[\beta$-CD + toluene + Fe(II)$]^{2+}$ in the gas phase, i.e., determine the favored binding configuration(s), we first consider the structure of the highly abundant $[\beta$-CD + Fe(II)$]^{2+}$ binary complex. As one of the first series of transition metal dications, Fe$^{2+}$ can be stabilized by forming 4, 5, 6 or even a higher number of coordination bonds. $\beta$-CD has many oxygens with lone pairs of electrons that can serve as donor electrons to Fe$^{2+}$. However, if Fe$^{2+}$ is situated on the outer surface or on the rim of the $\beta$-CD, it would not be able to form coordination bonds with $\beta$-CD oxygens in stable configurations such as those having tetrahedral, square pyramidal, trigonal bipyramidal, or octahedral geometries. Although Fe$^{2+}$ might adopt a square planar configuration with rim oxygens of $\beta$-CD, it would be atypical for Fe$^{2+}$ to interact with O donor electrons in such a configuration [34]. We propose that Fe$^{2+}$ is most likely situated inside the $\beta$-CD cavity near the lower (narrower) rim. In this orientation, Fe$^{2+}$ conceivably could form coordination bonds with nearby glucopyranosyl ring oxygens, glycosidic oxygens, and C(6) primary hydroxyl oxygens in stable configurations.

As for toluene, as previously mentioned, it cannot be observed by ES-MS bound to $\beta$-CD either inside or outside the cavity, even in the presence of added acid or alkali metal salts. Apparently, in order to maintain the ternary complex $[\beta$-CD + toluene + Fe(II)$]^{2+}$ in the gas phase, Fe$^{2+}$ must assist in binding toluene, via $d$-$\pi$ interaction, ion-induced electron interactions, and/or “crimping” of the cyclodextrin ring to strengthen van der Waals interactions. Conceivably, the configuration of the ternary complex could be one wherein Fe$^{2+}$ is located outside the $\beta$-CD rim and is sandwiched between the rim and the toluene molecule. Such a structure might resemble the geometry of the ternary complex shown schematically in Figure 3a. $[(C_6H_5CH_3)_2Fe]^{2+}$ (Figure 3b) is a reported dication and its synthesis can be found in the literature [35]. The formation and stabilization of $[(C_6H_5CH_3)_2Fe]^{2+}$ are favored in the condensed phase at low temperature ($-50^\circ$C) in aqueous methanol, with a high concentration of reagent Fe$^{2+}$ and toluene. However, neither ions corresponding to the structure shown in Figure 3b at m/z 120, nor those corresponding to that shown in Figure 3c at m/z 166 were observed in the ES mass spectrum of aqueous 1 mM Fe(ClO$_4$)$_2$ + toluene + 1 mM $\beta$-CD (see inset magnified range of m/z 110–180 in Figure 1b). Moreover, in a control experiment that examined 1 mM Fe(ClO$_4$)$_2$ aqueous solution with saturated toluene only (no $\beta$-CD), neither m/z 120 nor m/z 166 was observed. These facts cast doubt on the possibility that the ternary complex $[\beta$-CD + toluene + Fe(II)$]^{2+}$ would adopt the above mentioned sandwich structure (Figure 3a).

In considering the interacting species as pairs, it is apparent that the combination of ion-induced electron interactions and possible $d$-$\pi$ interactions between toluene and Fe$^{2+}$ are of insufficient strength to allow observation of $[\text{toluene} + \text{Fe(II)}]^{2+}$. Moreover, van der Waals interactions between toluene and the interior wall of the $\beta$-CD cavity are also not sufficiently strong to allow mass spectrometric detection of a protonated or sodiated inclusion complex in the gas phase. Because the probabilities are low for a stable complex to form wherein both Fe$^{2+}$ and toluene are situated outside the $\beta$-CD cavity, the favored configuration for binding of the $[\beta$-CD + toluene + Fe(II)$]^{2+}$ ternary complex is proposed to be that wherein both Fe$^{2+}$ and toluene are located within the cavity of $\beta$-CD. In this case, the ternary complex could be stabilized by Fe$^{2+}$-induced electron interactions, and conceivably Fe$^{2+}$ could even be forming coordination bonds with nearby oxygens from $\beta$-CD via $d$-lone pair electron interaction and with toluene via $d$-$\pi$ interaction at the same time.

Computational modeling tools have always been very useful in elucidating the structure of cyclodextrin inclusion complexes [36]. A molecular mechanics approach has been employed in this report to gain further insight into the structure of the ternary complex $[\beta$-CD + toluene + Fe(II)$]^{2+}$ via PC SPARTAN Plus version 2.0. To begin construction of the $[\beta$-CD + toluene + Fe(II)$]^{2+}$ complex, the $\beta$-CD structure was built first and optimized using the Energy Minimizer function of the PC SPARTAN program. Afterwards, Fe$^{2+}$ was added arbitrarily outside the lower (narrower) rim of $\beta$-CD, and toluene was added arbitrarily outside the upper
The Energy Minimizer was used again to optimize the three-component toluene/$\beta$-CD system. The obtained result showed that both Fe$^{2+}$ and toluene moved into the $\beta$-CD cavity to form a ternary inclusion complex. Equilibrium geometry optimization was then performed on the system at the MMFF94 level.

The final optimized ternary complex structure is displayed in Figure 4 (top, hydrogen atoms are not shown for improved clarity). This optimized structure indicates that Fe$^{2+}$ is surrounded by three C(6) primary hydroxyl oxygens of close proximity, with one ring oxygen, one glycosidic oxygen, and the phenyl ring of toluene, all at somewhat longer distances. In the lower portion of Figure 4 is a further simplified view of the final complex showing only Fe(II) and its nearest neigh-

(wider) rim of $\beta$-CD. The Energy Minimizer was used again to optimize the three-component toluene + $\beta$-CD + Fe$^{2+}$ system. The obtained result showed that both Fe$^{2+}$ and toluene moved into the $\beta$-CD cavity to form a ternary inclusion complex. Equilibrium geometry optimization was then performed on the system at the MMFF94 level.

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boring oxygen atoms and toluene in a somewhat twisted octahedral geometry, along with a listing of the distances between Fe\(^{2+}\) and the oxygens and carbons that may potentially be forming six coordination bonds. In comparing the obtained distances for the three primary hydroxyl oxygen-Fe(II) bond lengths (distance range = 2.13–2.22 Å), these compare favorably with literature values [37] for Fe-O bond lengths in coordinated alkylic alcohols (2.133 Å). On the other hand, literature values [37] for bond lengths in coordinated Fe-C complexes including cyclopentadienyl (2.080 Å), benzene (2.059 Å), and other arene complexes (2.101 Å) fall within a very narrow range that is much smaller than the shortest Fe-C distances (3.76 and 3.77 Å) obtained for the tolune complex shown in Figure 4. This discrepancy suggests that Fe(II) does not coordinate tolune. Instead, the orientation of the \(\pi\)-electron system shown in Figure 4 suggests that stabilization of tolune in the inclusion complex is achieved by ion interaction with the negative potential above the aromatic ring. While this negative potential has been calculated to have a maximum value at \(\sim\)1.5 Å [38], its influence extends well beyond this distance.

In addition to the above forces, van der Waals interactions between the inner wall of \(\beta\)-CD and tolune may also be strengthened through deformation or crimping of the cyclodextrin ring. Support for crimping comes from close inspection of the distances separating various atoms shown in Figure 4. For example, in comparing the distances between adjacent glycosidic oxygens connecting the glucose units comprising the \(\beta\)-CD ring, the shortest distance (3.893 Å) is that between the oxygen marked “b” and the nearest glycosidic oxygen to the left. The distance between this latter oxygen and the adjacent glycosidic oxygen to its left is the next shortest (4.273 Å). The other five analogous distances are all greater with the longest being 4.645 Å. The combination of electrostatic interactions and ring crimping can rationalize the experimentally observed stabilization of the [\(\beta\)-CD + tolune + Fe(II)]\(^{2+}\) complex. It should be noted that molecular mechanics methods have limitations in accurately determining exact system energies and interatomic distances. Therefore, a MMFF94 computation such as that shown in Figure 4 cannot completely rule out the possibility of Fe\(^{2+}\)-tolune coordination. Moreover, the structure shown in Figure 4 can only be considered to represent a local minimum on the MMFF94-generated potential energy surface. This structure may or may not be representative of the global minimum.

To provide further support for the above conclusions concerning the precise role of Fe(II) in stabilizing the complex comprised of \(\beta\)-CD and tolune, analogous experiments were performed using the Mg(II) dication in place of Fe(II). Ionic radii of Mg(II) (0.57 Å, 0.72, and 8 Å for coordination numbers of 4, 6, and 8, respectively) are very close to those of Fe(II) (0.63, 0.61, and 0.92 Å, respectively, for coordination numbers of 4, 6, and 8) [39]. The electrospray mass spectrum of the \(\beta\)-CD, tolune, and Mg(ClO\(_4\))\(_2\) mixture (Figure 5a) shows an intense peak corresponding to [\(\beta\)-CD + Mg(II)]\(^{2+}\) at \(m/z\) 579 as well as a peak corresponding to the ternary complex [\(\beta\)-CD + tolune + Mg(II)]\(^{2+}\) at \(m/z\) 625. Metastable decomposition and CID mass spectra of the \(m/z\) 625 precursor appear in Figures 5b and 5c, respectively. The CID spectrum showing uniquely the loss of tolune (Figure 5c) confirms the assignment of the ternary complex. The absence of metastable decompositions (Figure 5b) echoes the result for the Fe(II) ternary complex shown in Figure 2a, and attests to the strength of the dication-stabilized complex.

MMFF94 modeling of the Mg(II) ternary complex was undertaken in a manner strictly analogous to that performed for the Fe(II) complex. Upon optimization, [\(\beta\)-CD + tolune + Mg(II)]\(^{2+}\) appeared as an inclusion complex that had a form very similar to that obtained for the Fe(II) ternary complex. Five oxygens were present in very close proximity to the central Mg(II) dication in a geometry analogous to that shown in Figure 4 (points a-e), with the following distances: Mg\(^{2+}\)-a: 1.948 Å; Mg\(^{2+}\)-b: 2.456 Å; Mg\(^{2+}\)-c: 1.995 Å; Mg\(^{2+}\)-d: 2.296 Å; Mg\(^{2+}\)-e: 1.982 Å. The two carbons on the included tolune in closest proximity to Mg\(^{2+}\) were \(f_2\) (4.548 Å) and \(f_3\) (4.461 Å). Deformation of the \(\beta\)-CD ring was also evident with distances between adjacent glycosidic oxygens ranging from 3.912 to 4.587 Å. Because the charge of Mg(II) is the same as that of Fe(II), and the ionic radius is similar, the ensemble of experiments shown in Figure 5 in combination with the MMFF94 modeling further substantiate the rationalization of the additional stability afforded to the \(\beta\)-CD + tolune + dication complex through dication interaction and crimping of the cyclodextrin ring.

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) represents another type of aromatic compound that has both non-polar and polar moieties. Figure 6a is the ES mass spectrum of aqueous 1 mM \(\beta\)-CD + 1 mM Fe(ClO\(_4\))\(_2\) saturated 2,4,5-T. The ion at \(m/z\) 722 corresponds to the ternary complex [\(\beta\)-CD + 2,4,5-T + Fe(II)]\(^{2+}\). A CID experiment detecting product ions of the selected precursor at \(m/z\) 722 yields solely the product [\(\beta\)-CD + Fe(II)]\(^{2+}\) at \(m/z\) 595 (Figure 6b). This result confirms the composition of the initial ternary complex at \(m/z\) 722 to be [\(\beta\)-CD + 2,4,5-T + Fe(II)]\(^{2+}\). While it is possible that the ternary complex does not take the form of an inclusion complex (i.e., 2,4,5-T is situated outside \(\beta\)-CD), inclusion forms of the complex can obtain added stability via conformational strain relief on the \(\beta\)-CD ring [40–42]. Moreover, in a configuration where the trichlorophenyl ring of 2,4,5-T is inside the cavity, the guest molecule can be held by \(\pi\)-system interaction with the Fe\(^{2+}\) dication plus van der Waals interactions with the \(\beta\)-CD ring, possibly aided by Fe\(^{2+}\)-induced crimping of the \(\beta\)-CD ring, similar to those interactions proposed above for the [\(\beta\)-CD + tolune + Fe(II)]\(^{2+}\) complex. Alternatively, the polar part of 2,4,5-T, i.e., its carboxylic acid moiety, may be favored to enter into the
The β-CD cavity where it may preferentially interact with Fe²⁺.

In order to determine which part of 2,4,5-T, aromatic portion or carboxylic acid moiety, could be included in the β-CD cavity, m/z 850 (see Figure 6a) was further examined in a tandem mass spectrometry CID experiment. From Figure 6c, it can be seen that upon CID, the selected m/z 850 precursor yields m/z 722 corresponding to [β-CD + 2,4,5-T]²⁺ at m/z 104 and [Mg(II) + 3 toluene]²⁺ at m/z 150 are not observed; (b) metastable decomposition of selected m/z 625 precursor; (c) CID of m/z 625.

ES-MS experiments on a mixture of Fe²⁺ + α-CD + 2,4,5-T were performed to further probe the possible binding between Fe²⁺, 2,4,5-T and the smaller, six glucopyranose unit ring, α-CD. Figure 7a is the ES mass spectrum of aqueous 1 mM Fe²⁺ + 1 mM α-CD + saturated 2,4,5-T solution in which m/z 641 is proposed to be [α-CD + Fe(II) + 2,4,5-T]²⁺. A CID experiment on the selected m/z 641 precursor (Figure 7b) confirms that the ion is [α-CD + Fe(II) + 2,4,5-T]²⁺ which yields [α-CD + Fe(II)]²⁺ at m/z 514 upon decomposition. In
characterizing the \([\alpha\text{-CD} + \text{Fe(II)} + 2,4,5\text{-T}]^{2+}\) ternary complex, the size of the \(\alpha\text{-CD}\) cavity is too small for the aromatic trichlorophenyl ring of 2,4,5-T to fit inside. Instead, it is more likely that one carboxylic acid moiety of 2,4,5-T has entered into the \(\alpha\text{-CD}\) cavity. The fact that the ternary complex is observed despite the inability for the aromatic portion to fit within the cyclodextrin cavity supports the argument that for 2,4,5-T, its carboxylic moiety electron donor interaction with Fe\(^{2+}\) is strong enough to maintain the ternary complex in the gas phase. When the latter experiment was repeated using 2mM NaCl in place of Fe(ClO\(_4\))\(_2\), very low signals corresponding to \([\alpha\text{-CD} + 2,4,5\text{-T} + \text{Fe(II)}]^{2+}\) (m/z 647) and \([\alpha\text{-CD} + 2,4,5\text{-T} + 2\text{Na}]^{2+}\) (m/z 636) were observed. However, the singly charged analogs were not detectable, and neither were complexes containing two 2,4,5-T molecules.

Figure 6. Mass spectra of 1 mM \(\beta\text{-CD} + 1\) mM Fe(ClO\(_4\))\(_2\) saturated 2,4,5-T. (a) ES mass spectrum; (b) CID of m/z 722 precursor corresponding to \([\beta\text{-CD} + 2,4,5\text{-T} + \text{Fe(II)}]^{2+}\); (c) CID of m/z 850 precursor corresponding to \([\beta\text{-CD} + 2(2,4,5\text{-T}) + \text{Fe(II)}]^{2+}\).

Figure 7. ES mass spectra of 1 mM \(\alpha\text{-CD} + 1\) mM Fe(ClO\(_4\))\(_2\) saturated 2,4,5-T. (a) ES mass spectrum; (b) CID of m/z 641 precursor corresponding \([\alpha\text{-CD} + 2,4,5\text{-T} + \text{Fe(II)}]^{2+}\); m/z 514 represents \([\alpha\text{-CD} + \text{Fe(II)}]^{2+}\).
ion assignments at m/z 674 [α-CD + 2 (2,3-dihydroxynaphthalene) + Fe(II)]^2+, and at m/z 594 [α-CD + (2,3-dihydroxynaphthalene) + Fe(II)]^2+. For α-CD, the precursor ion at m/z 755 is assigned as [β-CD + 2 (2,3-dihydroxynaphthalene) + Fe(II)]^2+, and the precursor ion at m/z 675 is assigned as [β-CD + (2,3-dihydroxynaphthalene) + Fe(II)]^2+. CID results (Figure 8a, inset) confirm these assignments. For α-CD, precursor ion assignments at m/z 674 [α-CD + 2 (2,3-dihydroxynaphthalene) + Fe(II)]^2+, and at m/z 594 [α-CD + (2,3-dihydroxynaphthalene) + Fe(II)]^2+, are also confirmed by CID results (Figure 8c inset). The size of the β-CD cavity is suitable to include only one 2,3-dihydroxynaphthalene, while the second 2,3-dihydroxynaphthalene must be excluded from the CD cavity. The cavity of α-CD (with large rim inner diameter of 5.7 Å [10]), on the other hand, is too small to include even one 2,3-dihydroxynaphthalene. Thus, the quaternary complex [α-CD + 2 (2,3-dihydroxynaphthalene) + Fe(II)]^2+ cannot possibly be an inclusion complex. For either of the cycloextrin quaternary complexes, the second 2,3-dihydroxynaphthalene’s hydroxyl group(s) must be engaged in electrostatic interactions to stabilize the complex. When 2mM NaCl replaced Fe(ClO)4, neither α-CD nor β-CD produced detectable signals corresponding to [CD + 2,3-dihydroxynaphthalene + Na]^+. However, β-CD produced convincing signals for [β-CD + 2,3-dihydroxynaphthalene + 2Na]^+ (m/z 670), and [β-CD + 2(2,3-dihydroxynaphthalene) + 2Na]^+ (m/z 750). On the other hand, using α-CD, only a very low signal was observed at m/z 589 for [α-CD + 2,3-dihydroxynaphthalene + 2Na]^+. The above findings corroborate the conclusion that an inclusion complex of 2,3-dihydroxynaphthalene is formed only with β-CD.

The observed metastable decompositions of cycloextrin ternary and quaternary complexes with iron (II) and 2,3-dihydroxynaphthalene (Figure 8) further imply that non-inclusion forms of the complexes exist. The apparent ease of metastable decomposition contrasts with results obtained for the [β-CD + toluene + Fe(II)]^2+ (Figure 2a) and [β-CD + toluene + Mg(II)]^2+ (Figure 5b) ternary complexes where no metastable decompositions were observed. This contrast provides corroborative evidence that the latter two [β-CD + toluene + dication]^2+ complexes exist in the form of inclusion complexes. Moreover, compared to [β-CD + (2,3-dihydroxynaphthalene) + Fe(II)]^2+ (Figure 8b), [α-CD + (2,3-dihydroxynaphthalene) + Fe(II)]^2+ (Figure 8d) exhibits a marked increased tendency to undergo metastable decomposition. This is the trend that would expect if at least a portion of the guest 2,3-dihydroxynaphthalene molecule was inside the β-CD cavity. Nevertheless, it is clear that assigning an inclusion complex structure for a guest molecule bearing polar functional groups that interact with cycloextrin comes at some risk, even in the presence of metal ions such as Fe(II) or Mg(II) that can serve to stabilize the complex.

**Conclusion**

Novel gas-phase ternary complexes containing a Fe^2+ (or Mg^2+) dication have been characterized as specific inclusion complexes of the form [β-CD + toluene + dication]^2+ via ES-MS and ES-MS/MS experiments, as well as by a computational approach. Computational
studies indicate that, in the gas phase, the interaction between Fe(II) and the aromatic ring of toluene plays an important role in holding the toluene molecule inside the β-CD cavity. However, the distance between Fe(II) and the aryl carbons is significantly greater than one would expect for a coordinated complex. Further stabilization of the inclusion complex is likely achieved via deformation or crimping of the β-CD ring that strengthens the van der Waals interactions between the ring and toluene. If, in the ternary complex, toluene is substituted with another aromatic molecule that has both polar and non-polar parts (e.g., 2,4,5-T), the polar part of such molecules can interact with Fe(II) inside the CD cavity via (oxygen electron) electrostatic interactions, even when the non-polar part of the aromatic compound is situated outside the CD cavity. This means of complex formation between cyclodextrin, iron (II) and guest molecule in the gas phase may be different from that which is favored in aqueous solution where generally the non-polar part of a molecule would be driven into the CD cavity by hydrophobic forces. Moreover, the electrostatic interactions between the polar moiety of the guest molecule and the “rim” hydroxyl groups of cyclodextrin might stabilize a guest situated outside the CD cavity. Current investigations are underway to characterize potential ternary complexes comprised of cyclodextrin, aromatic molecules and other transition metal ions.

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