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Probing Cyclodextrin–Guest Associations Using Electrospray Mass Spectrometry

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Mass spectrometric investigation of noncovalent interactions has been made possible with the advent of electrospray ionization technique. Early electrospray studies of these interactions have typically examined biopolymers such as proteins and nucleic acids. Noncovalent interactions also exist between host cyclodextrins and various guest molecules. In this work, we use electrospray mass spectrometry to examine noncovalent host–guest associations between cyclodextrins and several organic molecules with the goal of identifying the types of interactions that predominate in the inclusion complexes formed. The importance of "mild" electrospray conditions and sample preparation procedures are discussed in reference to these studies. Ortho-, meta-, and para-nitrophenol are used as guest molecules with α, β and γ cyclodextrin hosts to study the effects of host size, guest substituent position, and host:-guest concentration ratio on complex formation. Relative binding affinities are determined for meta- and para-nitrophenol and agree with other solution phase studies. We also present the first report of complexes between the very hydrophobic polycyclic aromatic hydrocarbon pyrene and β-cyclodextrin as observed under "mild" electrospray conditions.

Keywords: Electrospray mass spectrometry, noncovalent interactions, cyclodextrins, polycyclic aromatic hydrocarbons, nitrophenols

INTRODUCTION

Direct mass spectrometric (MS) characterization of noncovalent complexes is useful in characterization of supramolecular structures and molecular recognition processes where methods with high specificity and sensitivity are needed. Until the recent development of electrospray ionization (ESI) [1] and matrix-assisted laser desorption (MALDI) [2], this task was typically out of the realm of MS capabilities. The introduction of ESI and MALDI has extended the capabilities of mass spectrometry to include the characterization of thermally labile molecules of high molecular weight (>100,000 daltons), such as proteins and oligonucleotides. Beyond molecular weight determination and elucidation of primary structures, ESI and MALDI have also been used to examine noncovalent interactions since these ionization methods impart only small amounts of internal energy to the desorbing ions [3]. Noncovalent associations between molecules are thus now routinely examined by mass spectrometry, particularly using ESI which

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is not significantly constrained by sample preparation requirements that often limit the utility of MALDI [3].

ESI is a technique where ions are generated by application of high voltage to a low flow of sample through a metal capillary. This results in formation of a plume of charged droplets in ambient air. As these droplets evaporate during their travel towards the inlet of a mass spectrometer, ionic species are generated. In general, ionic species in solution are transferred to the gas phase during the electrospray process, while neutrals tend to become ionized by adduction with other ionic species (metals, counter ions) or by electrochemical oxidation at the needle solution interface [4].

ESI is of particular interest because it results in formation of multiply charged ions in the gas phase with the charge distribution that can be characteristic of the three-dimensional structure of the macromolecules in solution. Furthermore, ESI can produce ions from solutions of widely varying composition, pH and ionic strength. This allows for the examination of molecules in different environments and comparison with other methods of condensed phase structure determination, such as nuclear magnetic resonance (NMR) spectroscopy [5]. ESI is therefore a technique that can be used to study noncovalent interactions between cyclodextrins (CDs) and various guest molecules exemplifying simple systems that display molecular recognition properties.

CDs are cyclic oligosaccharides used for enzyme modeling, chromatographic separations, drug microencapsulation, and other applications [6]. The most widely used CDs consist of 6, 7, and 8 glucopyranose units and are named α, β, and γ, respectively (Fig. 1). The hydrophilic exterior of these oligosaccharides renders them water soluble while their interior cavity is less polar than water, thereby allowing the CDs to accommodate guest molecules mainly on the basis of size and hydrophobicity [6, 7]. CD:guest complexation consists of a combination of hydrophobic, electrostatic, van der Waals, or sometimes hydrogen bonding interactions [6, 8]. It is useful to be able to identify which of these possible interactions persist or disappear during ESI examination of CD:guest complexes in order to realize the full utility of electrospray mass spectrometry (ESMS) for examination of such interactions.

In aqueous solutions, a variety of inclusion complexes are formed between CDs and organic molecules ranging from smaller alcohols and benzene derivatives to large polycyclic aromatic hydrocarbons (PAHs). These solution interactions have been studied using a variety of techniques such as, NMR, ultraviolet (UV) and fluorescence spectroscopy, chromatography and solubility measurements.

NMR spectroscopy has been used to determine binding constants of many different CD complexes. NMR studies are based on changes in the chemical shifts of the ^{1}H's and ^{13}C's in the CD and/or guest molecules that are observed upon complex formation [9]. NMR can be used to differentiate between CD inclusion complex formation and other CD:guest interactions that do not involve inclusion of the guest into the CD cavity [10]. However, NMR suffers from poor sensitivity. Optical spectroscopic properties are also altered as guest molecules are included into the hydrophobic CD cavity, thereby allowing the use of fluorescence or absorbance measurements to verify inclusion complex formation [11]. However, the utility of these techniques is limited by the characteristics of the guest molecule (*i.e.*, existence of a chromophore or fluorescence intensity).

Retention behavior of organic molecules in high performance liquid chromatography (HPLC) employing CD stationary phases or capillary electrophoresis (CE) utilizing CD additives is an indication of CD:guest interactions [12, 13]. Furthermore, CE has been used to determine binding constants for numerous CD:guest complexes [14, 15]. An advantage of these techniques over the previously mentioned

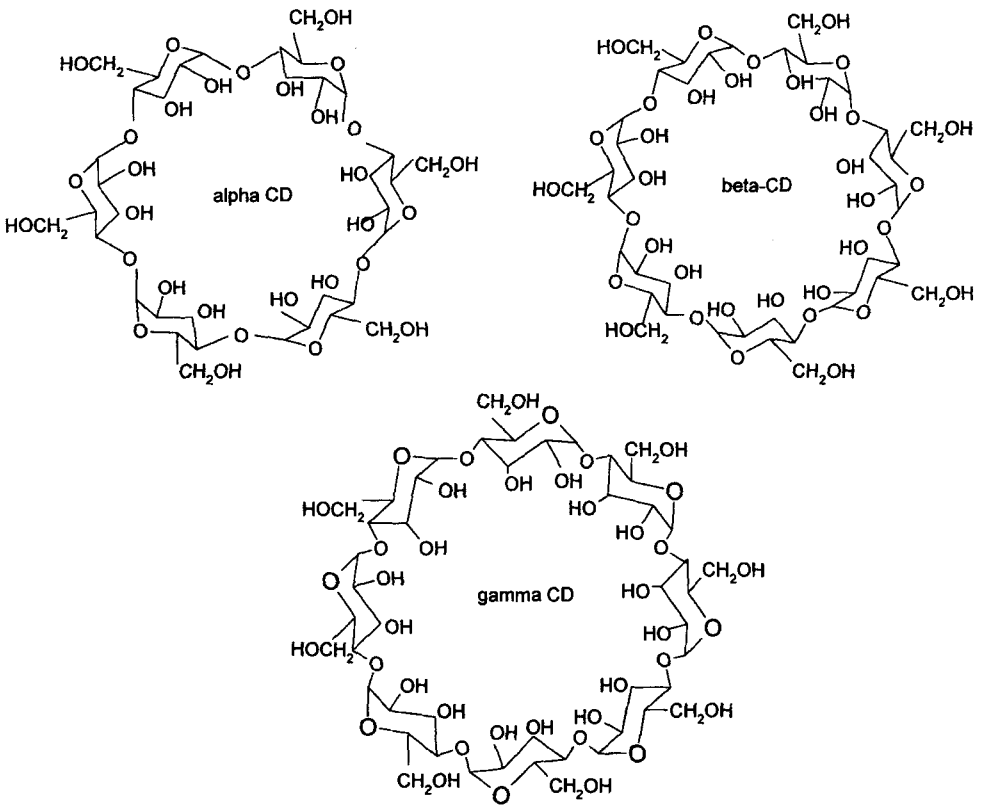

FIGURE 1 Depictions of native cyclodextrins (α, β, and γ).

spectroscopic methods is the ability to study a mixture of analytes simultaneously. However, these techniques also lack the specificity that can be acheived with an MS method.

As seen above, most of the information known about CD:guest complexes comes from solution phase studies, such as those mentioned. In ESMS, on the other hand, CD:guest interactions are studied in the gas phase. Gas-phase electrospray ions are thought to reflect species present in solution [16, 17]; however, as interfacial or gas phase processes can potentially influence the final product ions distribution, care is required in correlating ESI data to the equilibrium solution chemistry of these complexes. Some of these phase-based differences include nonspecific ion-dipole interactions between ions and molecules present in the highly charged ES droplets and hydrophobic interactions which do not persist in the gas phase. Also, ESI source operating conditions influence the mass spectra, thereby necessitating careful data analysis and adjustment of operating parameters. For example, Cunniff and Vouros state that many studies reporting the existence of noncovalent complexes between β-CD and various guest molecules (especially amino acids), as detected by ESMS, are incomplete. They suggest that many of the previous reports contain "false positives" that result from electrostatic interactions between the guest and the hydrophilic portion of the CD rather than actual inclusion of the guest molecule into the cavity of the CD [18]. However, they, and others, also present evidence that some CD:guest complexes formed in solution can be detected by ESMS [19]. For example, Penn *et al.*, detected gas-phase complexes of four peptides and permethylated-β-Cd by using ESI

[19]. They report that in temperature-induced dissociation and collision-induced dissociation studies, decomposition of the gas-phase complex varies in a fashion consistent with the formation constant for the complexes in solution. These results indicate that observation of gas-phase noncovalent complexes can provide relevant information about solution behavior. Finally, Lamcharfi *et al.* [20] questioned the role of ESI source equipment on MS ion spectral patterns of CD:guest complexes where, for some species, they had noted a dependence on the voltage at the end of the dielectric capillary (Analytica ESI source).

This work demonstrates the specificity of CD:guest interactions by examining complexation between (*p*-NP) and α-, β-, γ-CDs and then between α-CD and the three positional isomers of NP (*o*-, *m*-, and *p*-). The NPs are particularly suitable for our study because of their sufficient solubility, availability of stability constants and existence of X-ray crystallographic data showing how NP complexes α-CD. Furthermore, since they are positional isomers (same mass, same substituents), observed differences in complexing behavior can be attributed to selectivity of the association process rather than artifacts of ESI technique.

These studies are extended to examination of CD complexation by pyrene (Py) and 1-hydroxypyrene (Py-OH). For these multi-ring hydrophobic molecules, the "fit effect" is proposed to play a key role in gas phase detection of the CD:PAH complexes. However, complexes of pyrene and CDs are difficult to observe highlighting the significance of ESI source operating conditions and sample preparation methodologies.

RESULTS AND DISCUSSION

Complexes formed between CDs and guest molecules can be a result of total inclusion of the guest into the CD cavity (inclusion compounds). However, other associations between the CD and guest in which partial or no inclusion takes place are also common [6]. The latter is especially true when the relative dimensions of the guest and host cavity are not compatible.

The driving forces for complexation can arise from Coulombic, dipole–dipole, van der Waals and hydrogen bonding interactions between host and guest molecules or solvent effects including guest solvation and hydrophobic interactions. In solution, hydrophobic interactions favor complexation while solvation of the guest molecules hinders this process (because the guest species must shed their solvent shell to penetrate the nonpolar cavity of the host). Conversely, in MS, where ions are examined in the gas phase (no solvent), the relevant interactions are those that pertain to host and guest molecules (*e.g.*, size compatibility, dipole–dipole, H-bonding, electrostatic). In the absence of solvent, the CD-guest complexes have short lifetimes in the gas phase (depending on the rate of complexation and complex stability) especially if multiple strong interactions between the guest and host molecules are not present. Thus most of the MS-observed complexes of CDs deal with hydrophilic guest molecules capable of forming electrostatic interactions with the CD host [18]. However, this does not imply that any charged or hydrophilic molecule would randomly complex CDs in electrospray.

Size Effect: Interactions between *p*-NP and α-, β-, γ-CDs

In most discussions of CD complexation, it is stated that, in general, compounds containing one aromatic ring fit best into α-CD, two rings into β-CD, and three rings into γ-CD if bulky substituents are not interferring with inclusion. In these complexes, inclusion may be full or partial and the stability of the formed complexes depends on the depth of penetration. Also, the

"snugness" of the fit is important in determining the strength of the interaction. Typical stoichiometries observed are host:guest 1:1, 1:2, and 2:1 although the reported values possibly reflect the experimental conditions and methodologies employed.

ESMS examination of samples that were 50 μM in α-, β- or γ-CD, 50 μM in *p*-NP and 10 μM in NaI showed increasing complexation in the order $\gamma < \beta < \alpha$, consistent with the suggestion that α-CD and *p*-NP form the "best fit" and have the largest formation constants (438 L/mol for α-CD and 142 L/mol for β). The results of this experiment are shown in Table I (see Fig. 2 for a full scan mass spectrum of α-CD with *p*-NP). For α-CD, predominantly 2:1 host:guest complexes consisting of $[2CD:1NP:2Na]^{+2}$ and $[2CD:1(NP-H):3Na]^{+2}$ were detected at m/z 1065 and 1076 respectively, with minor amounts of the 1:1 CD:guest complex, $[1CD:1(NP-H):2Na]^{+1}$ (m/z 1156) and $[1CD:1(NP-H):3Na]^{+2}$ (m/z 589). Formation of complexes between the dissociated (NP-H) anion and α-CD reflects partial dissociation of *p*-NP in aqueous media. In fact, lowering the solution pH to approximately 4 with hydrochloric acid suppressed the complexes between (NP-H) and α-CD.

The identities of the CD:NP complexes were confirmed by collision activated dissociation (CAD). CAD is a powerful technique for structural identification of unknowns when the selected precursor ions fragment in a reproducible manner. In a typical CAD experiment with a triple quadrupole mass spectrometer, a precursor ion is selected with the first quadrupole. The precursor ion is then fragmented by collision with an inert gas, usually argon, in the second quadrupole or the rf-only quadrupole. Finally, the product ions of the dissociation are detected with the third quadrupole of the triple quadrupole mass spectrometer.

For the CAD studies, solutions that were 250 μM in *p*-NP or *m*-NP and 50 μM in α-CD were electrosprayed. The results were similar for each guest compound, and only those for *m*-NP are discussed here. The precursor ions m/z 1065 and 589, respectively corresponding to $[2CD:1NP:2Na]^{+2}$ and $[1CD:1(m\text{-}NP\text{-}H):3Na]^{+2}$, were examined. Dissociation of m/z 1065, shown in Figure 3a, at collision energies of 13 eV and collision gas pressure of 1.7 mTorr results in formation of a product with m/z 995 which corresponds to either $[CD:Na]^{+1}$ or $[2CD:2Na]^{+2}$. This corresponds to exclusion of the *m*-NP guest from the CD cavity as a result of collisions with argon in the rf-only quadrupole.

Dissociation of m/z 589, the α-CD complex with dissociated NP (Fig. 3b), results in appearance of a doubly charged ion with m/z 520 and corresponds to loss of a neutral NP molecule. This reaction requires exchange of a Na^+ for H^+. The proton is likely to be abstracted from the secondary CD hydroxyl groups at the wider end of the CD truncated cone, also known to catalyze the hydrolysis of phenyl esters by formation of an inclusion complex [21].

TABLE I Size Effect: Interactions between *p*-NP and α-, β-, γ-CD

Species	α-CD		β-CD		γ-CD	
	m/z	RA*	*m/z*	RA*	*m/z*	RA*
$[CD:Na]^{+2}$(Base Peak)	995	100	1157	100	1320	100
CD:guest complexes						
$[2CD:1NP:2Na]^{+2}$	1065	26.7	1227	8.0	1390	4.1
$[2CD:1(NP-H):3Na]^{+2}$	1076	4.1	1237	8.0	–	–
$[1CD:1(NP-H):2Na]^{+1}$	1156	2.0	1296	4.8	1461	3.6
Σ **RA****	–	32.8	–	20.8	–	7.7

* Denotes relative abundance.
** Denotes sums of relative abundances of the various CD:guest complexes.

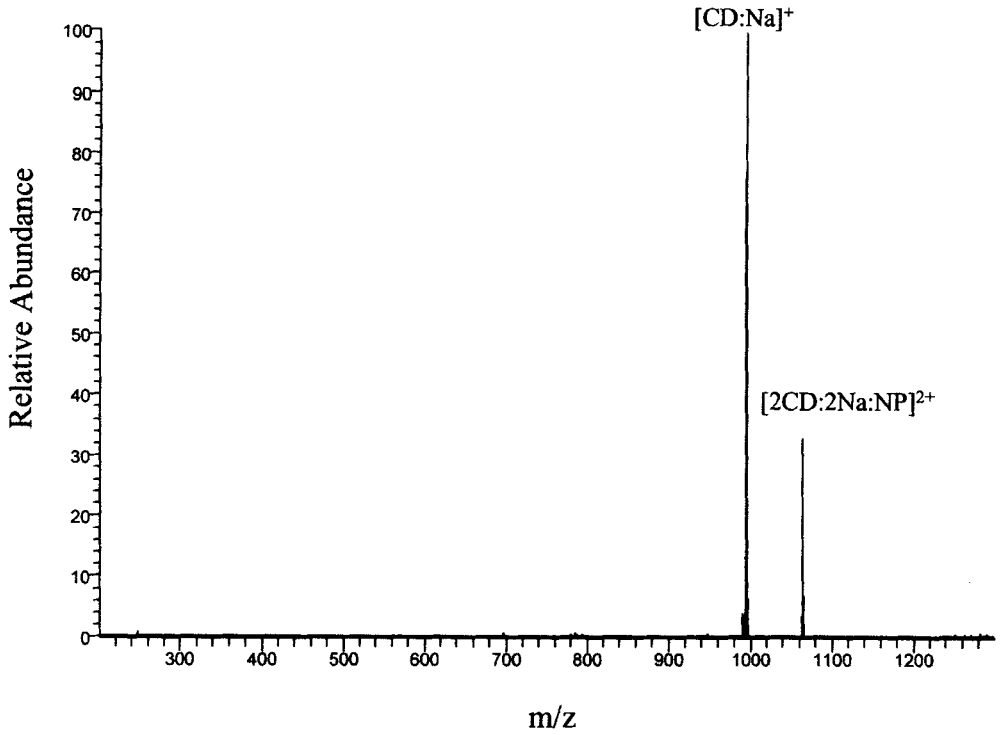

FIGURE 2 Full scan mass spectrum of 50 μm α-CD with 50 μm *p*-NP and 10 μm NaI.

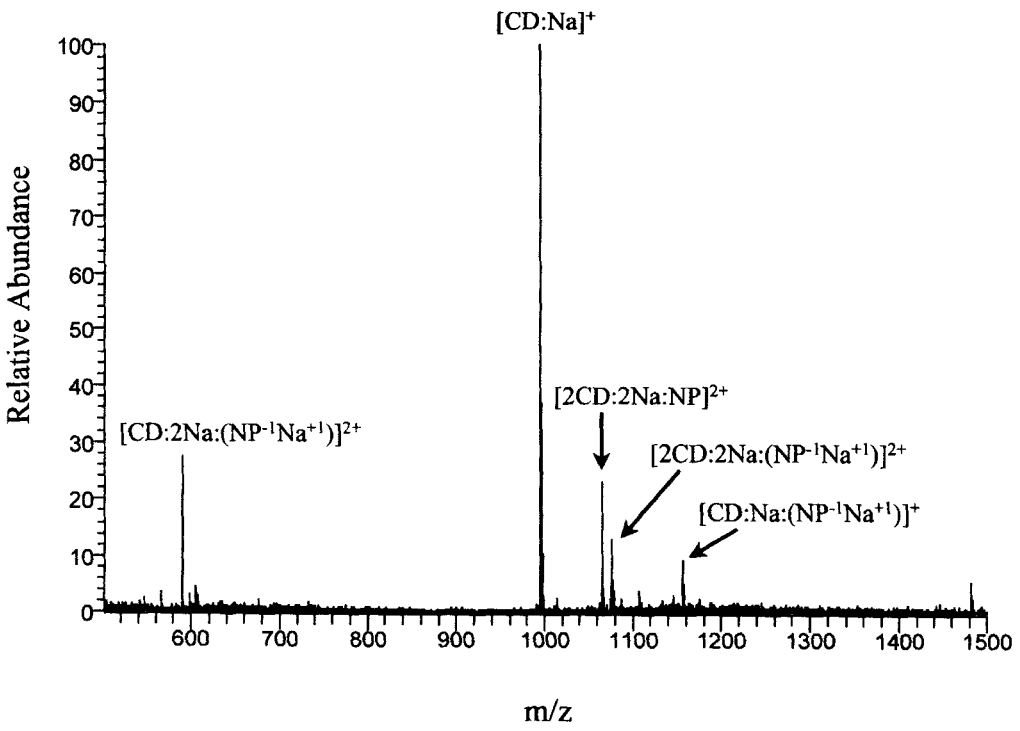

FIGURE 3a CAD spectrum of m/z 1065 $[2CD:2Na:NP]^{+2}$. Sample consists of 250 μm *m*-NP and 50 μm α-CD and 10 μm NaI. Collision cell pressure 1.7 mTorr argon; collision energy 13 eV.

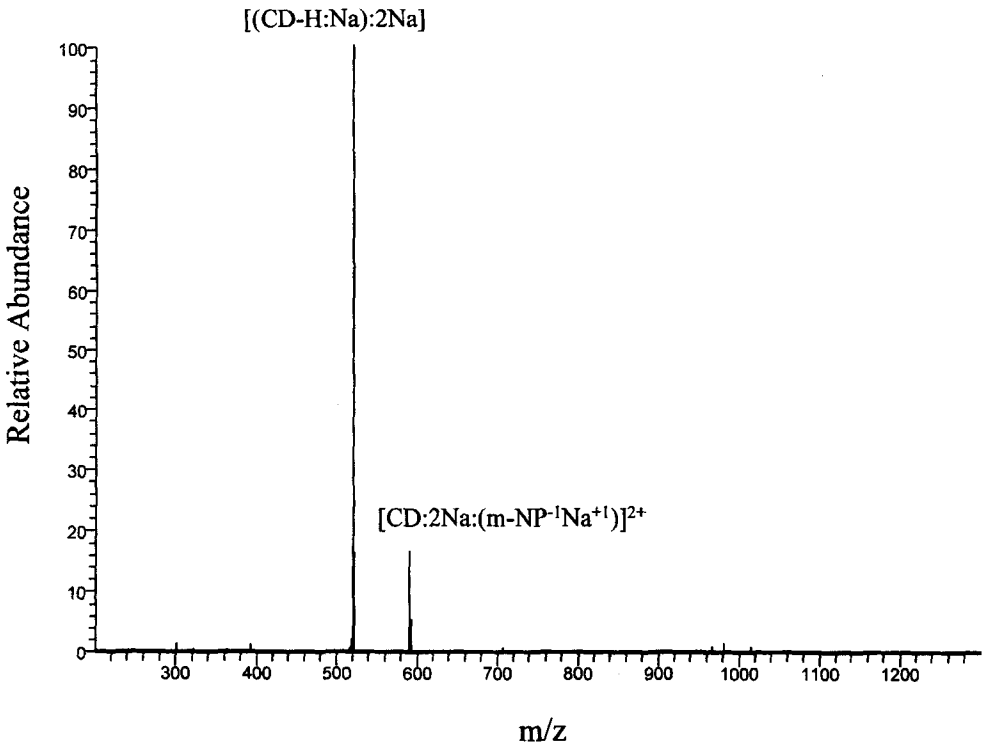

FIGURE 3b CAD spectrum of m/z 589 [CD:3Na:(*m*-NP)]$^{+2}$. Sample and CAD conditions same as in Figure 3a.

These CAD studies confirm the identity of the detected complexes; however, the spectra are not informative in a structural sense. Nevertheless, comparison of these tandem mass spectrometry results with those of other studies [22, 23] can be informative. For example, in these studies, the CD:guest complexes dissociated with the loss of a neutral guest molecule at lab frame collision energies of 13 eV or lower which translate into center-of-mass collision energies of approximately 0.5 eV when argon is the collision gas. However, the actual dissociation threshold is below this value because the mass spectrometer cannot be tuned accurately at lower collision energies; therefore, this was the lowest energy at which CAD spectra were obtained. This suggests that the CD:guest complex is not very strongly bound, particularly in comparison to the energies of dissociation noted by Penn and coworkers [19] for peptide:CD complexes (1–1.5 eV) and by Ramanathan and Prokai [23] for amino acid:CD complexes (0.9–2.9 eV). In both of these studies, dipole–dipole and electrostatic interactions play a significant role in the stability of the CD:guest complexes, which is also reflected in their CAD spectra that indicate proton transfer to and fragmentation of the CD hosts where peptides and amino acids are the guest. Such processes were not observed for the CD:NP complexes investigated here, since charge was carried by sodium ions. These experiments provided a rough measure of CD:guest complex stability when compared to other CD:guest CAD studies and suggested the presence of only weak interactions between the host CD and the guest molecule.

A noteworthy discrepancy between some of the solution studies and the ESMS data concerns the stoichiometry of the α-CD:NP complexes. In ESMS, the 2:1 stoichiometry was most promi-

nent, although small amounts of 1:1 complexes were also detected in equimolar samples of α-CD and *p*-NP. This is in contrast to literature reports of complexation between α-CD and *p*-NP which suggest a 1:1 stoichiometry using thin layer chromatography [24] and calorimetry [24]. Similarly, using electrophoretic methods [15], the reported stoichiometry of α-CD complexation with (NP-H) anion is 1:1 and that of β-CD with (NP-H) is 2:1.

To investigate how changes in relative host:-guest ratios would affect the observed stoichiometries of the α-CD: *p*-NP complexes in ESMS, a series of samples with host:guest ratios ranging from 1:1 to 1:10 (host:guest) were electrosprayed. The concentration of the 1:1 sample was 50 μM each for α-CD and *p*-NP and 10 μM NaI; other samples were prepared similarly, *e.g.*, 1:5 corresponded to 50 μM CD:250 μM *p*-NP. The results of this experiment are shown in Table II. Data show that sums of the absolute abundances of the 1:1 complexes ($[1CD:1(p\text{-NP-H}):2Na]^{+1}$ and $[1CD:1(p\text{-NP-H}):3Na]^{+2}$, m/z 1156 and 589 respectively) gradually increase with increasing concentrations of *p*-NP, while those of the 2:1 complexes ($[2CD:1NP:2Na]^{+2}$ and $[2CD:1(NP-H):3Na]^{+2}$, m/z 1065 and 1076) decrease. Thus, relative abundances of the 1:1 and 2:1 CD:guest complexes shift in a predictable manner when the ratio of CD to guest concentration is changed. However, the persistence of the 2:1 α-CD-guest complex at high NP concentrations is intriguing and raises the concern that the ESI process itself may be perturbing equilibria during the ion evaporation process. For example, a higher concentration of CD molecules at the surface of the evaporating drops could potentially shift the local equilibrium toward a 2:1 complex.

On the other hand, survey of CD:guest complexation literature raises the possibility that a mass spectrometrically detected 2:1 complex may not simply represent an artifact on ESI. For example, Armstrong and coworkers have suggested that incomplete insertion of *p*-NP in the CD cavity could lead to formation of 2:1 complexes. In this picture, one CD forms the inclusion complex while the other CD hydrogen bonds to the substrate near the cavity [12]. Such an effect is possible in solution (depending on [CD], ionic strength and solvent dielectric, *etc.*) and will be more pronounced in the gas phase in the absence of solvent, especially when the hydroxyl substituent of *p*-NP remains outside the CD cavity. Armstrong *et al.*, also note a preponderance of 1:1 reported stoichiometries in CD literature and suggest that a 2:1 stoichiometry may be more widespread than what literature indicates. Based on these reports and the above results, the 2:1 stoichiometry observed for NP and α-CD cannot be readily attributed to the ESI process as it could be actually reflective of solution chemistry. Understanding the degree to which ESI perturbs the equilibria for complex formation will require further research and will undoubtedly assist in the explanation of the ESMS observed stoichiometries.

TABLE II Stoichiometries of CD:guest complexes at various [α-CD]: [*p*-NP] ratios

Species	m/z	**1:1** AA*	**1:2** AA*	**1:5** AA*	**1:10** AA*
$[\alpha\text{-CD:Na}]^{+1}$(Base Peak)	995	4.0 e5	3.5 e5	3.7 e5	1.3 e5
1:1 CD:guest species $[1CD:1(NP-H):2Na]^{+1}$and $[1CD:1(NP-H):3Na]^{+2}$	1156, 589	8.0 e5	1.0 e7	1.41 e7	1.10 e7
2:1 CD:guest species $[2CD:NP:2Na]^{+2}$and $[2CD:1(NP-H):3Na]^{+2}$	1065, 1076	1.23 e7	1.46 e7	9.28 e6	3.71 e6

* Denotes absolute abundance of species detected by ESMS.

Substituent Effect: α-CD with *o-*, *m-*, and *p*-NP

Associations between α-CD and *o-*, *m-*, and *p*-NP were used to unequivocally demonstrate the specificity of host:guest interactions using ESMS. These molecules differ in the position of the hydroxyl substituent which determines the molecules' shapes thus influencing interactions with the CD cavity. For example, it is evident that *m*-NP is more sterically hindered than *p*-NP; hence it should have a smaller binding constant with α-CD.

Complexation between *o-*, *m-*, and *p*-NP and α-CD were studied under gentle electrospray conditions. Electrospray samples were 50 μM in α-CD and *o-*, *m-*, or *p*-NP and 10 μM in NaI. The complexes detected were $[2CD:1NP:2Na]^{+2}$ (m/z 1065), $[2\ CD:1(NP-H):3Na]^{+2}$ (m/z 1076) and $[1CD:1(NP-H):2Na]^{+1}$ (m/z 1156). As shown in Table III, complexation decreases in the order *p-* > *m-* > *o*-NP indicating a higher degree of complexation for the "more streamlined" *p*-isomer compared to the "sterically hindered" *o*-isomer. The decreasing order of complexation for α-CD with *p-* and *m*-NP is also consistent with reported formation constants determined by thin layer chromatography [24] (438 L/mol for *p*-NP and 105 L/mol for *m*-NP; no values were available for *o*-NP) and the crystallographic structures of CD:NP complexes [25]. Further, the complexes between CD and (NP-H) were more abundant for *p*-NP due to its lower pKa.

Relative Binding Affinities

Binding constants are often determined for CD:guest complexes to quantitate the interactions between CDs and guest molecules in solution. The equilibria involved in these associations are generally complex but it is possible to assume a certain stoichiometry for the complex, *e.g.*, 1:1, 2:1, *etc.*, and to calculate an apparent binding constant. Experimentally, complexation is measured for a set of samples where the host molecule is present in excess and the concentration of the guest species is varied incrementally.

A difficulty in using this procedure for determination of stability constants by ESMS is the multiplicity of charge states and differences in their desorption efficiencies. An alternate method takes advantage of the high specificity of MS methods for determination of relative binding affinities. For example, relative binding affinites for *m-* and *p*-NP and α-CD were determined in a single ESMS experiment. First, a sample that was 50 μM in both deuterated and non-deuterated *p*-NP, 100 μM α-CD and 100 μM NaI was examined by electrospray to test the effect of deuteration on the extent of complexation. The deuterated and non-deuterated NP:α-CD complexes were of equal abundance indicating no effect from deuteration on the extent of complexation.

Next a sample that was 50 μM in *m*-NP, 50 μM in deuterated *p*-NP (*p*-NPd_4), 100 μM α-CD and

TABLE III Host-guest interactions between α-CD and *o-*, *m-*, *p*-NP

Species	m/z	*o*-NP RA*	*m*-NP RA*	*p*-NP RA*
$[CD:Na]^{+1}$(Base Peak)	995	100	100	100
CD:guest complexes				
$[2CD:1NP:2Na]^{+2}$	1065	0.5	32.1	24.5
$[2CD:1(NP-H):3Na]^{+2}$	1076	1.5	4.5	20.8
$[1CD:1(NP-H):2Na]^{+1}$	1156	1.0	1.5	23.3
Σ **RA****	–	3.0	38.1	68.6

* Denotes relative abundance.
** Denotes sums of relative abundances of the various CD:guest complexes.

100 μM NaI was analyzed by ESMS. The CD:guest complexes detected were [2CD:1NP:2Na]$^{+2}$ (m/z 1065), [2CD:1(NP-H):3Na]$^{+2}$ (m/z 1076), [1CD:1(NP-H):2Na]$^{+1}$ (m/z 1156) and [1CD:1(NP-H):3Na]$^{+2}$ (m/z 589) for *m*-NP and [2CD:1NP:2Na]$^{+2}$ (m/z 1067), [2CD:(NP-H):3Na]$^{+2}$ (m/z 1078), [1CD:1(NP-H):2Na]$^{+1}$ (m/z 1160) and [1CD:(NP-H):3Na]$^{+2}$ (m/z 591) for *p*-NPd$_4$.

As shown in Table IV and Figure 4, relative host:guest binding affinities were significantly different for *m*-NP and *p*-NPd$_4$. With the exception of [2CD:1NP:2Na]$^{+2}$ species (CD complex with the undissociated NP), relative abundances of the *p*-NPd$_4$:α-CD complexes always exceeded those of the *m*-NP:α-CD complexes. This observation is reasonable in light of the dissociation of *m*- and *p*-NPd$_4$ in aqueous media resulting in [NP-H]/[NP] of ~3.8% for *p*-NP and 1.0% for *m*-NP. Thus the higher relative concentrations of undissociated *m*-NP relative to *p*-NPd$_4$ are reflected in the higher abundances of the [2CD:1NP:2Na]$^{+2}$ complex. Bertrand *et al.*, have reported that the ratios of $K_{equilibria}$ for the complexation of the (NP-H) anion relative to the undissociated NP were the same as the ratio of ionization constants of the uncomplexed NP [24].

CD Interactions with PAHs

The results thus far indicate that ESI can probe non-covalent interactions between nitrophenols and CDs, and it is clear that these interactions result from true host-guest interactions and are not false positives. It is difficult to understand why there should be any significant variation between the results for *p*-NP and *m*-NP, if the observation of these complexes is the result of non-specific association that occurs as a result of ESI. The fact that these types of interactions can be observed with NPs, where inclusion appears to be the driving force for complex formation, suggests that other inclusion driven complexes might be observed. A particularly interesting set of examples are PAH-CD complexes. PAHs form a group of compounds with documented mutagenic and carcinogenic properties [26]. Many bioremediation projects focus on developing materials that entrap these molecules in order to isolate, identify and quantify various PAHs present at an impacted site. In this regard, much effort has been expended towards developing CDs as additives in extractions and separations of PAHs [27].

A significant amount of literature is available concerning PAH interactions with CDs. For example, the stoichiometry of several different CD:PAH complexes and their binding constants have been determined by fluorescence measurements [28,29]. Warner *et al.*, reported the existence of 1:1 and 2:1 β-CD:pyrene complexes as a result of measuring the I/III intensity ratio of the pyrene monomer fluorescence in the presence of varying CD concentrations. They

TABLE IV Comparison of host:guest binding affinities for *m*-NP and *p*-NPd$_4$ with α-CD

Species	m/z	*m*-NP RA*	*p*-NPd$_4$ RA*
[CD:Na]$^{+1}$(Base Peak)	995	100	100
CD:guest complexes			
[2CD:1NP:2Na]$^{+2}$	1065/67	19.4	6.96
[2CD:1(NP-H):3Na]$^{+2}$	1076/78	9.99	54.9
[1CD:1(NP-H):1Na]$^{+1}$	1156/60	18.5	34.7
[1CD:1(NP-H):3Na]$^{+2}$	589/591	1.65	91.5
Σ **RA****	–	49.5	249.9

* Denotes relative abundance.
** Denotes sums of relative abundances of the various CD:guest complexes.

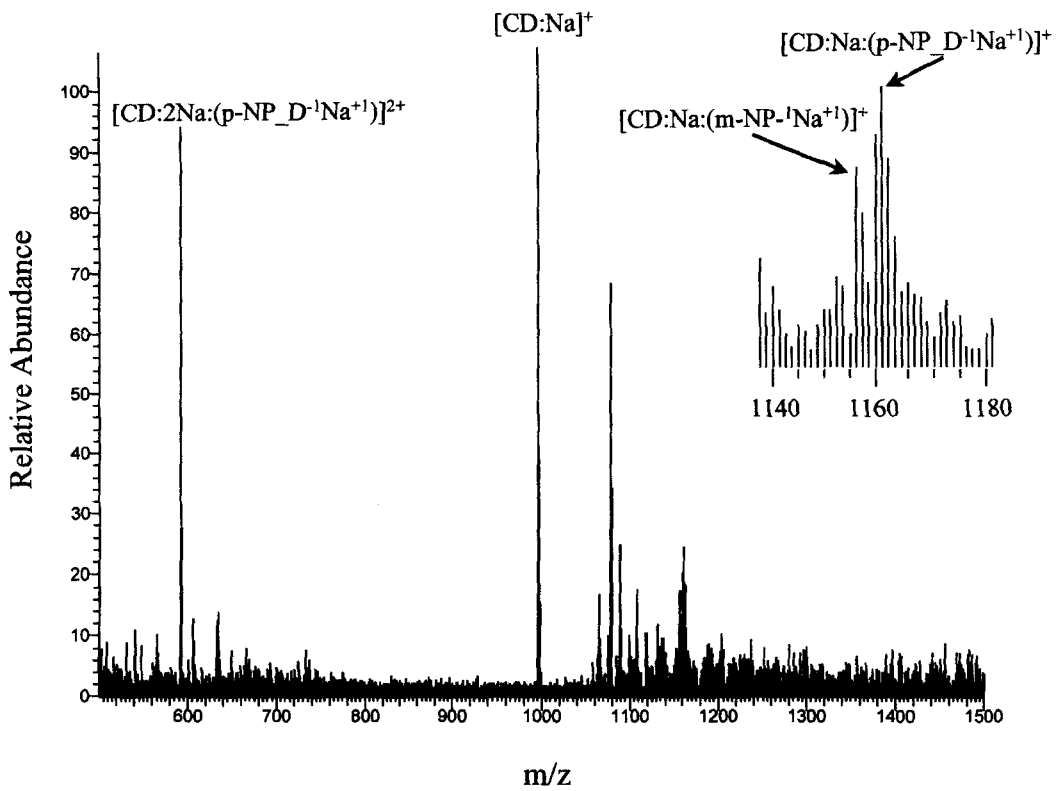

FIGURE 4 Mass spectrum of 100 μm α-CD and 100 μm NaI, 50 μm *m*-NP and 50 μm *p*-NPd_4.

report a binding constant for the 1:1 β-CD:pyrene complex of $8.53 \times 10^5 M^{-2}$ [28]. Furthermore, data from computer modeling studies of CD:PAH interactions (gas phase) are available for comparison with MS studies (also gas phase) [30].

Herein, the complexation behavior of pyrene (Py) and 1-hydroxypyrene (Py-OH) is examined by electrospray. Samples consisting of 200 μM β-CD and ~100 μM Py and Py-OH were electrosprayed under very "mild" operating conditions. Complexes detected were [2CD:1Py:2Na]$^{+2}$ (m/z 1259) for Py and [2CD:1Py-OH:2Na]$^{+2}$ (m/z 1266) for Py-OH. The relative abundances of these complexes were similar ranging from 5 to 8% of the base peak which was [CD:Na]$^{+1}$ (m/z 1157).

Mass spectrometric detection of a CD:Py complex is the first example of observing a CD inclusion complex where electrostatic interactions are essentially absent. As complexation in solution is largely driven by hydrophobic interactions, so in the gas phase the CD:Py complexes are not expected to be stabilized to any degree, because hydrophobic interactions are no longer relevant. In the absence of such interactions, detection of a gas phase CD:Py complex is proposed to indicate a "fit effect" showing encapsulation of Py by two CD molecules in a manner similar to that proposed by the computational studies [30]. This complex is very labile as evident from its facile dissociation in a CAD experiment where the collision offset was set to a minimum (−10 V) and only residual amounts of collision gas were present in the collision cell. (See Figs. 5a and b).

Another noteworthy observation was the relatively equal abundances of the CD:Py and

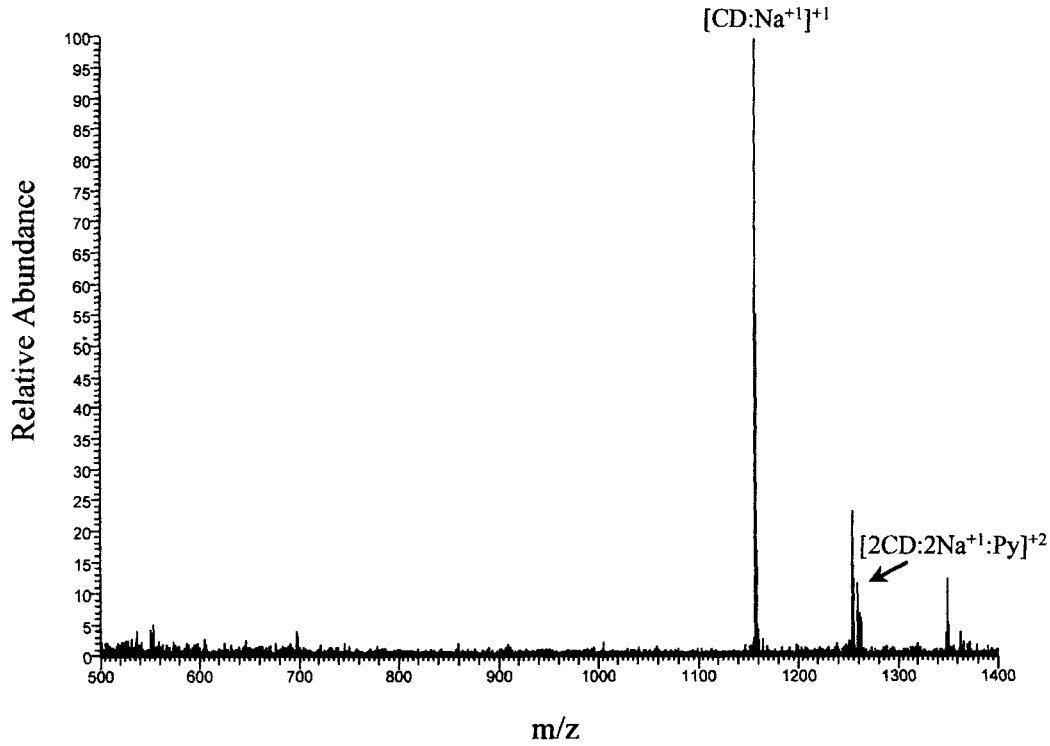

FIGURE 5a Mass spectrum of 100 μm Py and 200 μm β-CD.

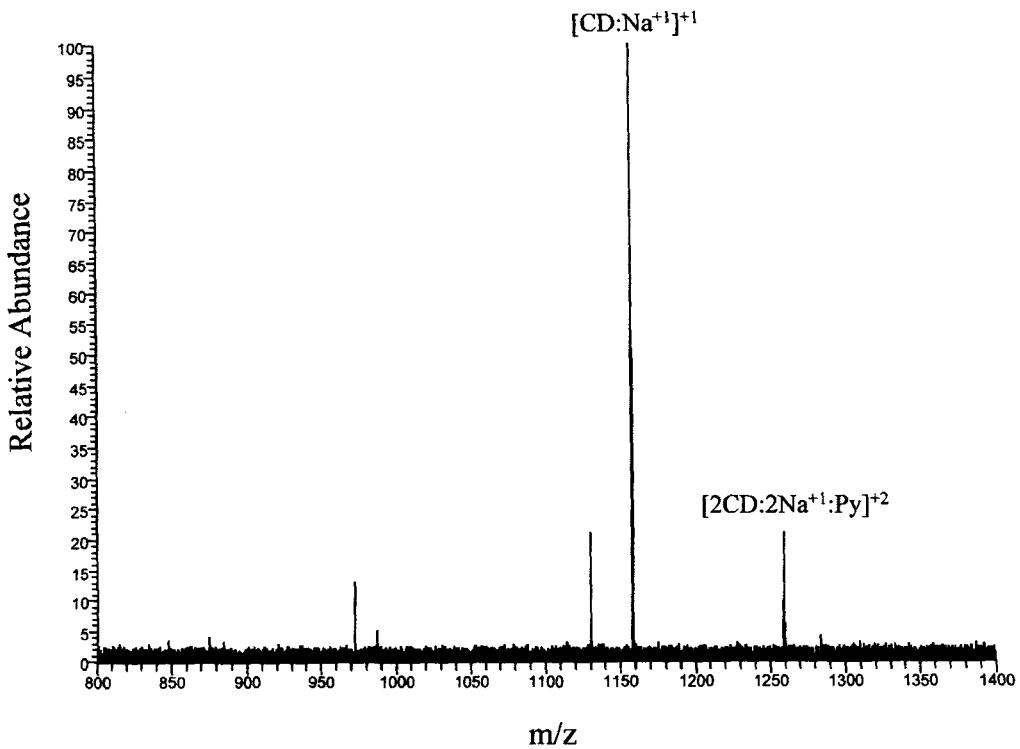

FIGURE 5b CAD spectrum of m/z 1259 $[2CD:2Na:Py]^{+2}$. Collision cell pressure 1.7 mTorr argon; collision energy ~9–10 eV.

CD:Py-OH complexes. This suggests that the polar substituent on the Py-OH does not influence complexation further complementing the notion that ESI results do not give false positives for all polar guest species (Py-OH). The CD:Py-OH complex was also confirmed by CAD. As with Py, CAD of the complex $[2CD:guest:2Na]^{+2}$ gave rise to m/z of 1157 corresponding to $[CD:Na]^{+1}$ or $[2CD:2Na]^{+2}$ and required low collision energies ($\sim$10 eV or less) demonstrating the lability of this species.

The trends discussed above were earlier reported for naphthalene, 1-naphthol, and 2,3-dihydroxy naphthalene [31]. For example, weak complexes were detected for each guest molecule with CD, the polar substituent on the guest molecule did not significantly enhance the relative abundances of the CD:guest complexes and low collision voltages dissociated the gas phase CD:guest complexes.

The significance of experimental conditions in detection of the weak CD:PAH complexes cannot be overemphasized. As stated in the **Materials and Methods**, the voltage difference between the capillary/tube lens, drying capillary temperature and absence of sheath gas were critical in detection of the weak CD:PAH complexes. The design of the ESI source equipment is also expected to be significant in preservation of weak noncovalent complexes. Presumably, some instrument designs will be more appropriate for investigations of gas phase CD:guest complexes.

MATERIALS AND METHODS

CDs and guest molecules were purchased from Sigma Chemical Co. (St. Louis, MO). All compounds were used without further purification except the nitrophenols (NP) which were recrystallized from 0.6 M HCl and ethanol. The electrospray samples were prepared by combining appropriate amounts of the CD and aqueous guest stock solutions and sonicating the mixture for 15 minutes prior to use. Each sample solution was spiked with NaI (final concentration = 10 μM) in order to improve electrospray sensitivity for detection of neutral CD:guest adducts. For highly insoluble guest molecules such as pyrene, 50 μL of methanol or acetonitrile was added to 1 mL of sample to prevent formation of microcrystalline precipitates.

Electrospray experiments were performed on a TSQ-70 triple quadrupole mass spectrometer (Finnigan-MAT Corp., San Jose, CA) fitted with an electrospray interface and a laboratory-made "nano-ESI" source. The mass spectrometer was initially tuned and calibrated by electrospraying a standard solution of 20 pm/μL tetrapepetide methionyl-arginyl-phenylalanyl-alanine (Sigma) and 5 pm/μL myoglobin (Sigma) in 1:1 methanol:0.25% acetic acid. Following tuning and calibration, the commercial electrospray needle assembly was replaced with the laboratory made nano-ESI source which was constructed after the design of Wilm and Mann [32]. The nano-ESI source was used in the complexation studies because it affords easy ESI of purely aqueous solutions without the use of a nebulizing gas (which was found to disrupt some weak CD:guest associations). For nano-ESI, glass needles pulled to a fine tapered 2–5 μm tip (World Precision Instruments, Sarasota, Fl) were made electrically conductive by vacuum depositing 40 nm of chromium and 250 nm of gold along one side. The nano-ESI needles were filled with approximately 15 μL of the aqueous CD-guest samples and inserted into an insulated holder placed several millimeters away from the ESI heated capillary inlet.

Experiments were run under "mild" ESI conditions of +2 to 2.5 kV high voltage, drying capillary temperature of 180°C and capillary/tube lens ΔV of 30 to 40 V. A nebulizing gas and liquid sheath were not used. It is important to note that ΔV values affect ion transmission to the mass analyzer and strongly correlate with the scanned mass range. Since high values of ΔV, required for transmission of high mass ions,

also result in "in-source" fragmentation of molecular ions, ESI sensitivity was sacrificed by minimizing ΔV to create very "gentle" conditions in the ESI interface.

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