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Probing molecular recognition by mass spectrometry

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Abstract

The use of mass spectrometry for the study of host–guest complexation and molecular recognition involving either synthetic hosts or biological hosts has been a growing area of research over the past decade. Mass spectrometry has allowed the first studies of host–guest chemistry in a solvent-free environment in which both size-selectivity and electronic effects influence the formation, reactions and stabilities of gas-phase host–guest complexes. Aspects of solution equilibria, such as the determination of binding selectivities of hosts and binding constants, may be examined by using electrospray ionization to transfer noncovalent complexes from solution to the gas phase for analysis. This article will review some of the highlights involving the application of mass spectrometry for solving problems in the area of molecular recognition. (Int J Mass Spectrom 200 (2000) 57–69) © 2000 Elsevier Science B.V.

Keywords: Molecular recognition; Host-guest chemistry; Electrospray ionization; Complexation

1. Introduction

Molecular recognition is a central theme of many important biological and chemical phenomena, including enzyme catalysis, biological regulatory functions, drug actions, ion transport, antibody–antigen association, cellular recognition, signal induction by neurotransmitters, translation and transcription of the genetic code, and energy transfer [1]. One of the central concepts of molecular recognition is the issue of "selectivity," i.e. the preferential binding of one guest over another by the host. It is this aspect that has propelled the applications of molecular recognition into one of the most promising strategies for designing molecules for specific purposes, such as the design of ion sensors, chelating agents that can be used to extract toxic metals from waste water, and artificial hosts that mimic biological receptors [1]. From a developmental standpoint, the evaluation of binding selectivities and the measurement of binding constants are key for establishing structure/selectivity relationships, rationalizing mechanisms of selective complexation, and ultimately applying the principles of molecular recognition to design ligands with targeted binding properties. The use of mass spectrometry for the study of host–guest complexation and molecular recognition involving either biological hosts or synthetic hosts has been an enormously active area of research over the past decade (for related reviews see [2–5], and the development of electrospray ionization (ESI) [6] has cemented the future of mass spectrometry in this important area of science. This article will focus on some of the key questions in

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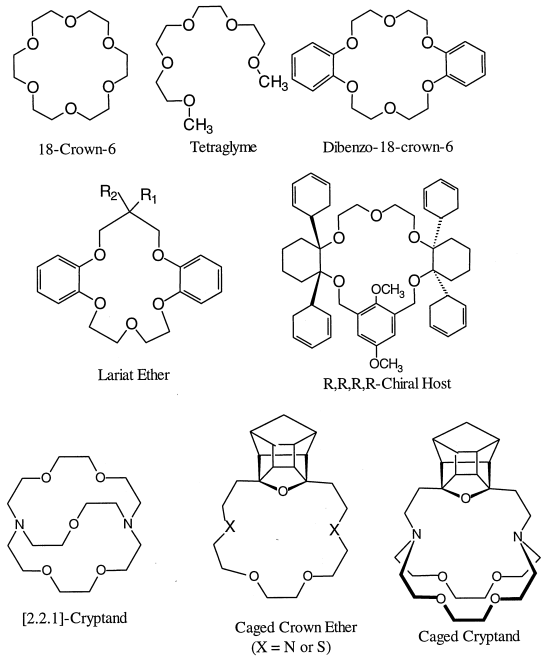

Fig. 1. Structures of hosts.

the area of molecular recognition that have been elegantly probed by mass spectrometry.

2. Host–guest chemistry in the gas phase

The early 1990s was a seminal period for the study of molecular recognition by mass spectrometry, in which the formation, reactions, and dissociation of host–guest complexes were studied in a gas-phase environment, thus allowing access to the intrinsic properties of the host–guest complexes because of the absence of solvent effects. Highly specific and selective ion chemistry of the host–guest partners was explored extensively by using various mass spectrometric methods. Most of these early studies involved simple synthetic hosts (Fig. 1, top row) and simple guests such as alkali metal ions or ammonium ions. The most frequently studied hosts in both solution and in the gas phase have remained the landmark synthetic crown ethers, first described by Pederson in 1967 [7]. Related macrocycles, such as substituted crowns and cryptands, and acyclic polyethers, such as glymes,

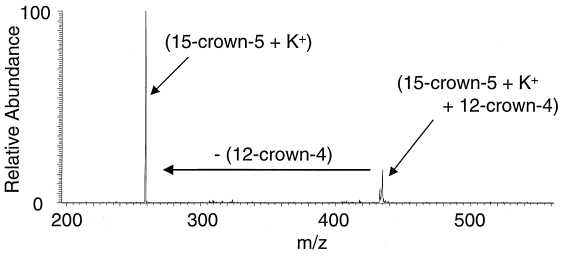

Fig. 2. Collisional activated dissociation mass spectrum of the (12-crown-4 + K^+ + 15-crown-5) complex formed by ESI and analyzed by using a Finnigan LCQ-Duo mass spectrometer.

have also been the targets of numerous gas-phase studies (Fig. 1).

One of the first examples of the reactions between hosts and guests in a totally gas-phase environment was reported in 1991 by the Brodbelt group in which complexes between perfluorinated crown ether anions and molecular oxygen were formed by ion–molecule reactions in the source of a triple quadrupole mass spectrometer [8]. This study catalyzed interest in the examination of other types of host–guest reactions in the gas phase [9–19]. The Brodbelt group extensively used a dissociation method coined “the kinetic method” [20] to probe the favored fragmentation pathways of 2:1 host:guest complexes in which two different hosts were simultaneously bound to a single guest ion. Both acyclic polyether hosts (i.e. glymes) and cyclic hosts (i.e. crown ethers) were used in these studies, and the guests ranged from simple alkali metal ions to a variety of ammonium ions. The 2:1 dimer-type complexes were formed by fast atom bombardment or by way of gas-phase ion–molecule association reactions and then subjected to collisional activated dissociation. The orders of the relative guest affinities of the hosts within the 2:1 sandwich complexes were assigned based on the preferential retention of the guest ion by either host in the complex upon dissociation. The example shown in Fig. 2, involving formation, isolation, and collisional activated dissociation of (12-crown-4 + K^+ + 15-crown-5), indicates that the complex dissociates exclusively by loss of 12-crown-4, a result that reflects the greater K^+ affinity of 15-crown-5 over that of 12-crown-4. It was found that these types of size

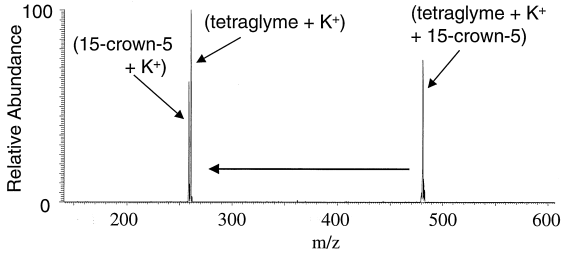

Fig. 3. Collisional activated dissociation mass spectrum of (tetraglyme + K^+ + 15-crown-5) complex complex formed by ESI and analyzed by using a Finnigan LCQ-Duo mass spectrometer.

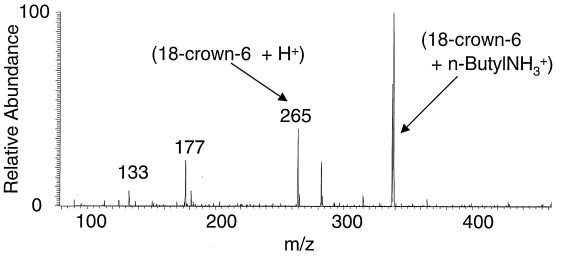

Fig. 4. Collisional activated dissociation mass spectrum of (18-crown-6 + *n*-butyl-NH_3^+) complex complex formed by ESI and analyzed by using a Finnigan LCQ-Duo mass spectrometer.

selective trends were more notable for the smaller guest ions (such as Li^+ and Na^+) than the larger guest ions (such as Rb^+ and Cs^+) [9,13]. It was also shown that the cyclic polyethers possessed greater guest ion affinities than the corresponding acyclic analogs [13]. For example, the complex (15-crown-5+ K^+ + tetraglyme) dissociates by loss of either 15-crown-5 (the favored pathway) or by loss of tetraglyme (the acyclic analog of 15-crown-5) to a lesser extent, indicating that tetraglyme exhibits a greater affinity for K^+ relative to that of 15-crown-5 (see Fig. 3).

Ammonium ion/host complexes were also studied in detail by using dissociation methods and ligand exchange methods [11,12, 14–16]. The dissociation patterns of many of the complexes, such as that observed for $(18\text{-crown-6} + NH_4)^+$, were striking because the complexes did not simply disassemble by cleavage of the hydrogen bonds to form the host and guest counterparts [15]. Instead, in many cases the protonated host underwent extensive cleavages of the macrocyclic skeleton, thus suggesting that the orignal host–ammonium ion complexes were strongly bound by an array of intermolecular hydrogen bonds. An example of this behavior is shown in Fig. 4 for the collisional activated dissociation of (18-crown-6 + *n*-butyl-NH_3^+). The complex dissociates by loss of *n*-butylamine, an expected loss, but the fragment ions at *m/z*177 and 133 result from losses of two or three C_2H_4O units from the 18-crown-6 host, dissociation pathways that involve covalent bond cleavages and thus require substantial energy. Since a resonant collisional activation process was used for these experiments, only the mass-selected precursor is activated, meaning that the formation of the fragment ions at *m/z*133 and 177 stem directly from energization of the precursor complex. This finding confirms that the complex is so strongly bound by intermolecular hydrogen bonds that dissociation by cleavage of covalent bonds of 18-crown-6 is competitive with cleavage of the hydrogen bonds between the amine and 18-crown-6. In fact, dissociation energies of a series of polyether/ammonium ion complexes were measured by monitoring the thresholds for fragmentation in a quadrupole ion trap [19]. For example, the critical energy for dissociation of crown ether/NH_4^+ complexes ranged from 32 kcal/mol for 12-crown-4 to 35 kcal/mol for 15-crown-5 to 41 kcal/mol for 18-crown-6. The highest energies were noted for complexes containing 18-crown-6 and larger ammonium ions like *n*-butylammonium ion, for which a value of $>$50 kcal/mol was obtained.

Dearden's group has comprehensively explored the formation and reactions of alkali metal complexes containing polyethers, such as crown ethers or acyclic analogs (glymes), by using Fourier transform ion cyclotron resonance (FTICR) mass spectrometry [21–30]. In many of these superb studies, polyether/alkali metal complexes were generated from ion–molecule association reactions between laser-desorbed metal ions and volatile ethers [21–23,29]. In one series of studies, a polyether/alkali metal complex was isolated in the gas phase and allowed to react with neutral polyether molecules, resulting in formation of 2:1 polyether:metal complexes. The formation rate constants for the 2:1 polyether:alkali metal complexes

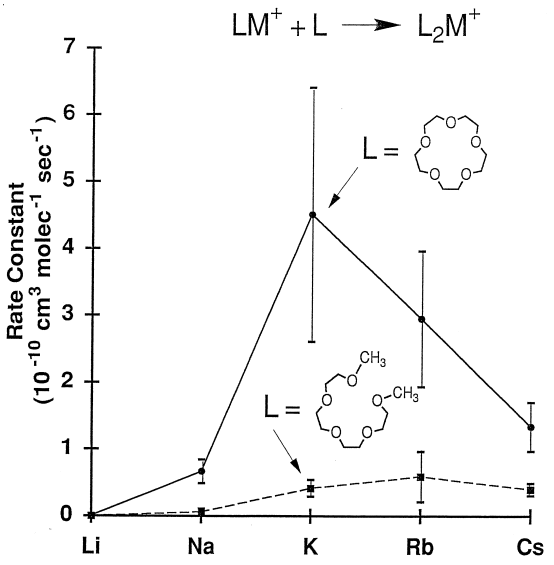

Fig. 5. Rate constants for formation of alkali metal cation-bound dimers of 15-crown-5 and tetraglyme in a FTICR mass spectrometer. Reprinted with permission from American Chemical Society [22].

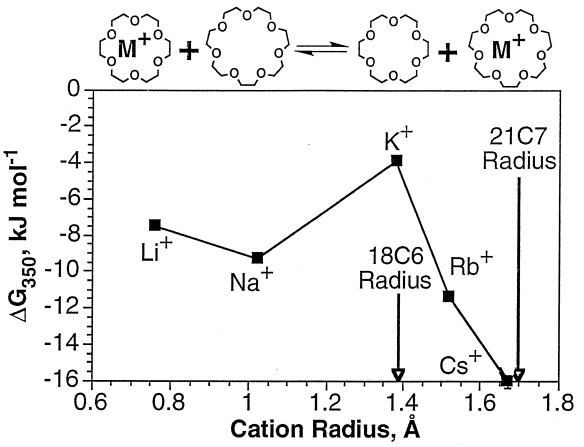

Fig. 6. Δ*G*values derived from the equilbrium constants for transfer of alkali metal cations from 18-crown-6 to 21-crown-7 in the gas phase, assuming 350 K in a FTICR mass spectrometer. Reprinted with permission from American Chemical Society [23].

incorporating cyclic ethers were generally found to be an order of magnitude greater than those for the corresponding acyclic ethers, as shown in Fig. 5 for the rate constants for formation of alkali metal-bound dimer complexes of 15-crown-5 versus its acyclic analog, tetraglyme [22]. This was the first evidence for a "macrocyclic effect" in the gas phase [22]. Moreover, the cyclic ethers demonstrated considerable size selectivity in the rate constants for formation of the 2:1 dimer complexes, where, for example, the 12-crown-4 ligand had the highest rate constant for reactions with Na^+, as compared to the 15-crown-5 ligand, which had the highest rate constant for reactions with K^+. Reactions involving the transfer of an alkali metal ion from one polyether to another showed that larger polyethers had stronger affinities for the metal ions than smaller polyethers, as shown in Fig. 6 for the alkali metal transfer reactions involving 18-crown-6 and 21-crown-7. Determination of the equilibrium constants for transfer of the various alkali metal cations from 18-crown-6 to 21-crown-7, along with estimation of the ΔG values for the reactions illustrated that the transfer reactions, although exoergic in every case, also reflected a degree of size selectivity in the gas phase [23]. In fact, the transfer reaction involving K^+, a metal ion that most closely matches the cavity size of 18-crown-6, was least exoergic, whereas the most exoergic reaction involved Cs^+, a metal ion that most closely matches the cavity size of 21-crown-7 (Fig. 6). The capabilities of FTICR mass spectrometry for the measurement of gas-phase rate constants or equilibrium constants for transfer of guest ions between hosts or formation of host–guest complexes has also been extended to studies involving cryptands [28], calixarenes [29], and cyclodextrins [30].

Armentrout's group has determined the binding energies of gas-phase alkali metal/ether complexes in detail by using a guided ion beam tandem mass spectrometer to measure thresholds of dissociation of mass-selected complexes [31–37]. It was found that the bond dissociation energies of the alkali metal ion complexes increased as the size of the crown ether increased, for example going from 1.96 eV for (12-crown-4 + K^+) to 2.12 eV for (15-crown-5 + K^+) to 2.43 eV for (18-crown-6 + K^+) (see Table 1). Moreover, the results also confirmed that the binding energies of crown ether/alkali metal complexes in the gas phase correlated primarily with the charge density of the metal ion. For instance, the bond dissociation

Table 1
Bond energies (eV) for crown ether alkali metal complexes[a]

Host	Li^+	Na^+	K^+	Rb^+	Cs^+
12-crown-4	3.85	2.61	1.96	0.96	0.88
15-crown-5	NA	3.05	2.12	1.18	1.04
18-crown-6	NA	3.07	2.43	1.98	1.74

[a] Reprinted (with permission) in part from [37].

energy decreased to 1.18 eV for the (15-crown-5 + Rb^+) complex relative to the 2.12 eV value obtained for the analogous K^+ complex, due to changes in the magnitude of the charge-dependent electrostatic interactions.

The capability for evaluating aspects of chiral molecular recognition by mass spectrometry has been explored by several groups [38–45], initially by using fast atom bombardment methods and then by electrospray ionization, a softer ionization method in which excitation, which could disrupt or alter the host–guest interactions that are key for chiral selectivity, was minimized. The chiral recognition of enantiomeric guests by hosts has been correlated with the intensities of the corresponding diastereomeric host–guest complexes observed in the mass spectra. Because the enantiomeric guests have identical molecular weights, typically one of the guests is isotopically labeled with deuteriums to cause a mass shift in one of the diastereomeric host–guest complexes. This method has been used to estimate the chiral recognition abilities of substituted crown ether hosts for enantiomeric amino esters [41,42] or naphthyl ammonium ions [40] and spiroacetal polyethers for enantiomeric phenylglycine methyl esters [43]. For example, using this enantiomer labeled guest method, it was shown that the R,R,R,R-host shown in Fig. 1 exhibited a greater complexation selectivity for an R amino ester guest by a factor of 1.5 over the analogous S guest [42]. Using a related method, the equilibrium constants for the transfer of enantiomeric ammonium ions bound to chiral dimethyldiketopyridino-18-crown-6 to 18-crown-6 [44], as well as the transfer of neutral enantiomeric amines between protonated crowns [45], were determined in a FTICR instrument. The equilibrium constants yielded the free energy changes, thus allowing estimation of the stabilities of the different host–guest complexes. In one of the studies, it was found that dimethyldiketopyridino-18-crown-6 bound one enantiomer of α-(1-naphthyl)ethylamine more strongly by 3.5 ± 0.6 kJ mol^{-1} over the other enantiomer [45].

3. Host–guest chemistry in solution

3.1 Measurement of binding selectivities by electrospray ionization mass spectrometry

Advances in the construction of novel hosts and applications of supramolecular chemistry have created a great need for new analytical techniques that can characterize binding selectivities or binding constants with minimal sample consumption. Binding constants of host–guest complexes and selectivities of hosts in solution can be obtained from many conventional methods [46], including calorimetry, potentiometry, spectrophotometry, and nuclear magnetic resonance (NMR) titrimetry. The limitations of these, including limited sensitivity, limited solvent compatibility, and lack of structural information, have highlighted the need for mass spectrometric strategies for probing aspects of molecular recognition in solution.

Electrospray ionization mass spectrometry allows the study of a wide variety of host–guest complexes and other noncovalent complexes formed in solution because the process is gentle enough to allow the survival of many types of weakly bound complexes [6]. Although the electrospray ionization mass spectrometry (ESI-MS) process involves a large change in the bulk solution environment caused by solvent evaporation and corresponding changes in the localized concentration of species, some features of the original equilibrium of the solution may be retained in the types and distribution of species in the gas phase. The distribution of species observed in the gas phase after ESI is influenced by the surface activity and relative evaporation rates of ions. For example, ions that have lower solvation energies typically have higher surface activities and thus are more easily generated in the ESI process, giving higher ion intensities, whereas ions with larger solvation ener-

gies may remain in the droplets that are not as efficiently sampled by the mass spectrometer. For ions with different structures, solvation energies, charges, and hydrophobicities, the proportion that become gaseous ions may vary greatly, leading to discrimination in the intensities of species observed in the resulting mass spectra [6]. In one of the first studies, it was found that the intensities of alkali metal ions sprayed from an aqueous solution correlated inversely with their solvation energies (i.e. Cs^+ giving the greatest intensity and Li^+ giving the lowest intensity), clearly showing the active role that desolvation plays in the ESI process [47]. To overcome the problems associated with different ESI efficiencies, ESI "response factors" for different species should typically be evaluated and weighted into any type of quantitative measurement involving ESI-MS. Although the concerns about whether ESI mass spectra reflect the equilibrium of species formed in solution remain heated, there are many cases in which the correlation between the solution distribution and the mass spectral distribution of ions is excellent. For this reason, there have been a growing number of mass spectrometric studies that have probed aspects of host–guest chemistry in solution by using ESI to transfer the complexes from the natural solution environment for analysis in the gas phase [47–58]. Moreover, because of the shortcomings associated with conventional methods for probing aspects of molecular recognition, such as NMR, potentiometry, extraction, or ultraviolet–visible spectroscopic methods [46], ESI-MS has moved to the forefront of new analytical methods for evaluating quantitative aspects of host–guest chemistry, probing structures of complexes, and verifying stoichiometries of complexes in solution.

One of the first studies to evaluate the correlation between host–guest complexation in solution and the resulting ESI-MS of the solutions was reported by Leize et al. in 1996 [47]. The distribution of (18-crown-6 + Na^+), (18-crown-6 + K^+), (18-crown-6 + Rb^+), and (18-crown-6 + Cs^+) complexes in a methanol/water (70:30) solution was calculated based on the known binding constants of the crown ether complexes and compared to the ESI-MS intensities of the corresponding complexes. The agreement was very good, with the percent distribution agreeing within 10% for all complexes [47]. Similar good agreement was obtained for a solution containing the same four alkali metal ions and a 2.2.2 cryptand. This study clearly showed that ESI-MS could be used to probe the thermodynamic equilibrium of species present in solution. In a comparison of the distribution of (18-crown-6 + Na^+) and (18-crown-6 + K^+) complexes in a methanolic solution, Gokel and Wang likewise found that the ESI-MS intensities correlated well with the distribution predicted based on direct analysis of the methanolic solution by ion-selective electrodes [48]. Significant differences in the ESI efficiencies of different complexes containing the same host were not found for either of these first studies, thus there was no evidence for large variations in the response factors. This uniformity in response factors was attributed to the fact that the solvation energies of the complexes were likely similar because the metals were bound in the cavities of the macrocycles. Liu and co-workers examined the alkali metal complexation of a series of lariat ethers in methanol by ESI-MS and found that for these complexes, variations in the spray efficiencies occurred as the size of the metal ion changed, thus requiring calibration of the ESI-MS intensities to account for the variations [49,50]. However, the ESI-MS method was found to be a convenient way to evaluate binding selectivities of the synthetic lariat ethers. This method has also been used to evaluate the ammonium ion selectivities of calixarene capsules [51].

The use of ESI-MS to study binding selectivity in solution has been extensively investigated by the Brodbelt group [52–58], with the aim of determining the validity and applicability of using ESI-MS as an alternative to conventional methods for measuring aspects of solution equilibria. A series of model hosts, including crown ethers and related analogs, and numerous simple guests, including alkali metal ions and ammonium ions, were used in these studies. Solutions containing defined quantities of two hosts and one guest or one host with two guests were analyzed by ESI-MS. The intensities of the complexes observed in the mass spectra were integrated and recorded as a

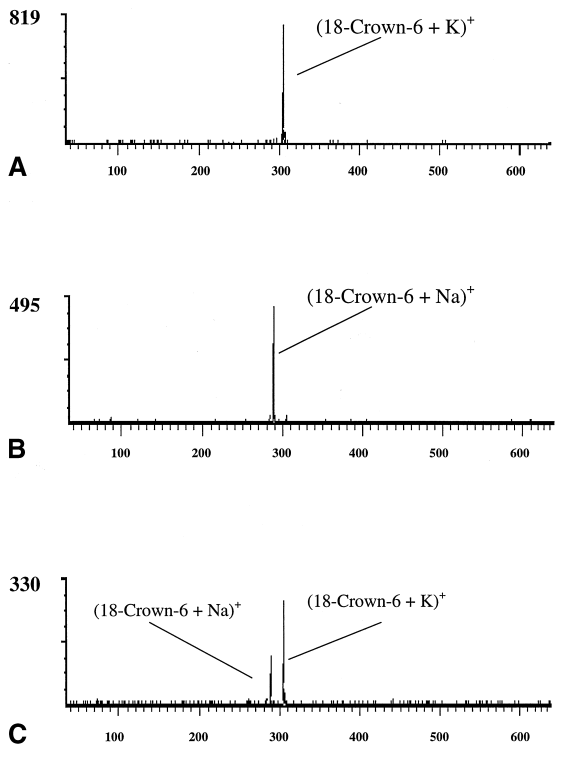

Fig. 7. ESI-MS of 18-crown-6 with (a) K^+ (1:1), (b) Na^+ (1:1), and (c) Na^+ and K^+ (2:1:1) in methanol, in which the concentration of 18-crown-6 is 1.5×10^{-4} M, by using a quadrupole ion trap mass spectrometer. Reprinted with permission from Elsevier Science [52].

"selectivity ratio," indicating the intensity of one host–guest complex relative to that of another. Then comparisons were made between the theoretical distributions of host–guest complexes in solution derived from known binding constants and the known initial concentrations of host and guest components.

An example of the general method is shown in Fig. 7. Fig 7(a) shows the ESI spectrum for a methanol solution containing 18-crown-6 at 1.5×10^{-4} M and potassium chloride at 1.5×10^{-4} M. The expected concentration of the (18-crown-6 + K^+) complexes in methanol is calculated as 1.4×10^{-4} M based on the known binding constant of (18-crown-6 + K^+) complex ($\log K = 6.08$ from [59]). In Fig. 7(a), the magnitude of the (18-crown-6 + K^+) complex is represented by 819 intensity units, based on the peak area. Fig. 7(b) shows the ESI-MS for the analogous solution containing 1.5×10^{-4} M each of 18-crown-6 and sodium chloride. The concentration of (18-crown-6 + Na^+) complexes in methanol is calculated as 8.7×10^{-5} M based on the known binding constant of (18-crown-6 + Na^+), ($\log K = 4.35$ from [59]). The intensity of the (18-crown-6 + Na^+) complex is 495 units. The intensities obtained for these two 1:1 mixtures scale with the calculated concentrations of the complexes in solution, indicating that these two complexes have similar ESI efficiencies. If the observed intensities for the two solutions did not scale with the calculated concentrations, then a response factor or "correction factor" would be used to scale the intensities. As shown in Fig. 7(c), the selectivity of 18-crown-6 obtained from the ratio of intensities of (18-crown-6 + K^+) to (18-crown-6 + Na^+) is 2.1 (i.e. preference for K^+ over Na^+) which agrees well with the ratio calculated based on the distribution of complexes predicted from the known binding constants (i.e. selectivity ratio = 2.0).

In general, solvation was found to play an important role in the ability to quantify binding selectivities by ESI-MS. The more similar the solvation energies of the two complexes in the mixture, the more quantifiable their binding selectivities were by ESI-MS, and there was less need for detailed calibration procedures to normalize the ESI response factors of different types of complexes. Optimum results are obtained when ESI mass spectra are collected for solutions containing only a single host and guest in conjunction with the ESI-MS for solutions containing mixtures of hosts and guests, thus permitting the correction of ESI response factors and normalization of spectral intensities. The ability of ESI-MS results to predict solution equilibria distributions was best for cases in which binding selectivities of a single host for different guest ions, rather than the competition of multiple hosts for a single guest, were monitored. The latter cases involve large differences in ESI efficiencies because the solvation energies of the resulting host–guest complexes vary greatly as the hosts change.

The hosts that have been studied in detail using this type of ESI-MS strategy include 18-crown-6, 15-crown-5, 12-crown-4, dibenzo-18-crown-6, dicyclo-

Table 2
Selectivity ratios for Distribution of K^+ vs. Na^+ complexes[a]

	Theoretical equilibrium ratio of complexes: (Host + K^+)/(Host + Na^+)	ESI-MS ratio of complexes: (Host + K^+)/(Host + Na^+)
18-crown-6/K^+/Na^+[b]		
2:1:1 in CH_3OH	2.0	2.0 ± 0.1
1:1:1 in CH_3OH	8.1	7.3 ± 0.1
1:2:2 in CH_3OH	29	36 ± 2
1:5:5 in CH_3OH	43	45 ± 4
2:1:1 in CH_3CN	2.1	2.2 ± 0.2
2:1:1 in $CHCl_3$	∞	9.2 ± 0.2
1:2:2 in H_2O	17	18 ± 1
15-crown-5/K^+/Na^+		
1:5:5 in CH_3OH	3.8	4.2 ± 0.1
Dibenzo 18C6/K^+/Na^+		
1:5:5 in CH_3OH	1.5	1.3 ± 0.1
Azo-18C6/K^+/Na^+		
1:5:5 in CH_3OH	21	26 ± 2

[a] The theoretical equilibrium ratios are found by dividing the concentration of the (host + K^+) complex by the concentration of the (host + Na^+) complex. The concentrations are calculated by solving a system of simultaneous equilibrium equations using reported binding constants as described in the test. All binding constants were obtained from [60]. Reprinted (with permission) in part from [54].

[b] The ESI solutions contained 1.5×10^{-4} M of the 18-crown-6 analog, and 7.5×10^{-5} M of each NaCl and KCL in solution. All values ±0.3 and taken from [54].

[c] The ESI experiments incorporated 3% methanol.

hexano-18-crown-6, and 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane, all involving complexation of alkali metal ions, ammonium ions, or transition metal ions. Their complexation in several different solvent systems has also been examined, ranging from aqueous to organic. A summary of some of the results obtained for these hosts is shown in Table 2, tabulated as K^+/Na^+ selectivities based on the intensities of complexes in the ESI-MS and compared to the equilibrium distribution of complexes in solution. Good agreement is found in most cases, thus allowing rapid determination of binding selectivities of hosts in a variety of solvents by ESI-MS.

Because ESI is compatible with many solvents, it allows examination of host–guest complexation in a range of solvents by a single analytical method. Alkali metal binding selectivities obtained in methanol, acetonitrile, and chloroform are reported in Table 2 for 18-crown-6, and the influence of the solvent environment is evident. As the polarity of the solvent increases, the K^+/Na^+ binding selectivity decreases. This trend stems from the increasing solvation energy of the alkali metal ions in more polar solutions, thus decreasing the ability of 18-crown-6 to desolvate either metal ion and suppressing the binding selectivity.

After evaluation of some of the uses and limitations of the ESI-MS method, the method was used to explore the binding selectivities of several arrays of synthetic hosts, including a series of dibenzo-16-crown-5 lariat ethers [56], a group of caged crown ligands [58], and several calixarenes [55,57]. In one study, the alkali metal complexation of a series of dibenzo-16-crown-5 lariat ethers containing methoxy, carboxylic acid, ester, or amide pendant groups was studied based on ESI-MS examination of solutions containing one lariat ether with three alkali metal ions (Fig. 1, Table 3) [56]. Most of these lariat ethers studied were found to be selective for Na^+ versus either Li^+ or K^+ in methanolic solution. Three factors

Table 3
Alkali metal selectivities of lariat ethers measured by electrospray in chloroform-methanol (1:19) solutions[a]

R_1	R_2	% $[LE + Li]^+$	% $[LE + Na]^+$	% $[LE + K]^+$	Na^+/K^+
H	OCH_2COOH	0	79	22	3.6
C_3H_7	OCH_2COOH	0	87	13	6.7
H	OCH_2COOEt	60	40	0	∞
C_3H_7	OCH_2COOEt	77	22	1	22
H	OCH_2CONMe_2	0	60	40	1.5
C_3H_7	OCH_2CONMe_2	0	84	16	5.3
H	OCH_2CONH_2	0	73	26	2.8
C_3H_7	OCH_2CONH_2	0	80	20	4.0

[a] The standard deviation is ±7% of the listed number. Reprinted (with permission) from [56].

contributed to the observed alkali metal ion selectivities of the lariat ethers: the cavity sizes of the lariat ethers, solvent effects that affected the formation of perching (i.e. metal above the cavity) versus nesting (i.e. metal within the cavity) complexes, and the basicities of the side arms which influenced the ability of each lariat ether to extract metal ions from the solution. For example, the lariat ether with the least basic side arm (i.e. the ester sidearm) preferentially bound the Li^+ cation, whereas the lariat ethers with the carboxylic acid and amide side arms preferred Na^+. It was also shown that the inclusion of a second pendant group, i.e. the propyl group, increased the binding selectivity, as expected based on the ability of the propyl group to reinforce the optimum binding configuration of the lariat ether.

The ESI-MS method was also used to screen the heavy metal binding selectivities of five caged crown ethers (see Fig. 1) [58]. The binding preferences for Hg^{2+}, Pb^{2+}, Cd^{2+}, and Cu^{2+} were obtained rapidly and with minimal sample consumption which was vital for the small amounts of ligands available for analysis. Most of the cage compounds preferentially complexed Hg^{2+}, except for the cage cryptand derivative, which favored Pb^{2+}. The favorable positioning of the nitrogen or sulfur atoms promoted optimal linear coordination of Hg^{2+}, whereas the cryptand derivative favored Pb^{2+} because of its larger cavity size. This study also showcased the importance of counterion effects on complexation. The counterions of the metal salts affected the type of complexes observed in the ESI-MS because the strengths of the metal–anion bonds influenced retention of the anion in the complexes. For instance, diaza-15-crown-5 preferred to bind mercury over the slightly smaller metals, copper and cadmium, based on the ESI-MS of solutions containing metal perchlorate or nitrate salts, but it preferred copper and cadmium over mercury in solutions containing metal chloride salts. The larger, symmetrical anions, such as the perchlorates and nitrates, allowed for the negative charge to be more delocalized, thus creating a weaker ion pair between the counterion and metal. When the chloride formed a strong ion pair with the metal ion, the nominal size and coordination geometry of the guest changed relative to that of a free metal or weak ion pair that dissociated in the ESI process. Thus, the coordination of the smaller metals by diaza-15-crown-5 was more favorable for the metal chloride experiments.

ESI-mass spectrometry has also been used in several studies to estimate binding selectivities or relative binding affinities in biochemical systems [61–70], such as receptor–ligand complexes like protein–drug [61,63], DNA–drug [62,65,68,70], protein–peptide [67], RNA–drug [69], or peptide–RNA [66] noncovalent complexes. Most of these studies have entailed using ESI-MS to analyze solutions containing one receptor (i.e. the protein or the DNA molecule) and one or more ligands (i.e. drugs). The intensities of the complexes were used to qualitatively estimate the binding preferences of the receptor. One of the first reports involved the complexation of an immunophilin protein with different immunosuppressive drugs, such as rapamycin [61]. Integration of the peaks corresponding to the noncovalent complexes formed in solutions containing one protein with two

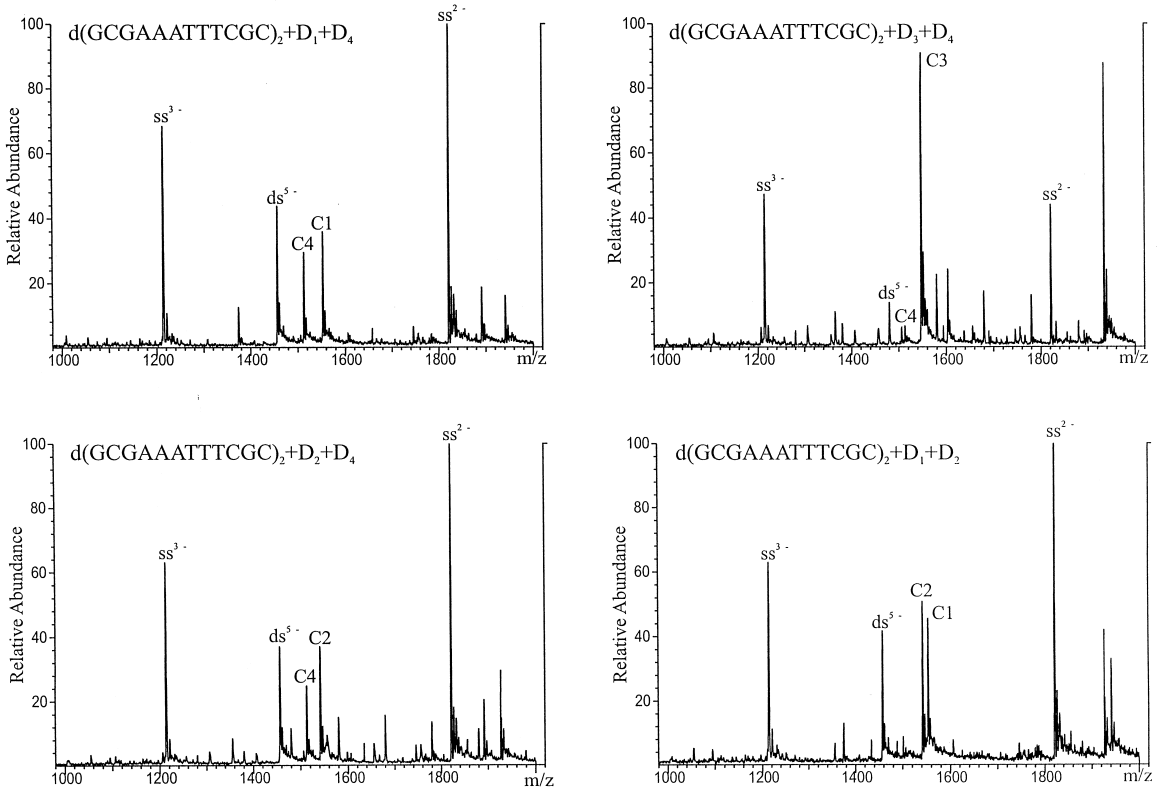

Fig. 8. ESI-MS of solutions containing a duplex and two drugs (D1–D4), resulting in duplex-drug complexes labeled as C1–C4, by using a Finnigan LCQ mass spectrometer. Reprinted with permission from American Chemical Society [70].

drugs, indicated that the complexation of rapamycin was favored over that of other analogs. This competition strategy has also been used to evaluate the binding affinities of single strand and duplex oligodeoxynucleotides (models of DNA) to various classes of drugs that are known to form noncovalent complexes with DNA via binding to the minor groove or intercalation, such as distamycins and anthracyclines [62,65,66,70]. An example is shown in Fig. 8 for the competitive binding of four drugs (where D1 is distamycin, D2 is Hoeschst33258, D3 is Hoeschst33342, and D4 is berenil) with a duplex [70]. Based on the intensities of the duplex/drug complexes (labeled as C1–C4 in the mass spectra to designate which drug is bound to the duplex), the order of binding preferences was determined to be D3 > D2 > D1 > D4. In a variation of the competitive binding experiment, the abundances of unbound duplexes to bound duplex-drug complexes in the ESI-MS were used to estimate the relative binding affinities of these same duplexes [70]. These types of experiments, which required less than a nanomole of sample, provided a rapid, efficient, accurate way to assess binding affinities in biochemical systems.

3.2. Measurement of binding constants by ESI-MS

Quantitative aspects of host–guest complexation are determined by measurement of binding constants (often given as log K values for the complexation equilibrium), dissociation constants, or formation constants, in which the equilibrium reaction is represented by

$$H + G \overset{K}{\rightleftharpoons} HG$$

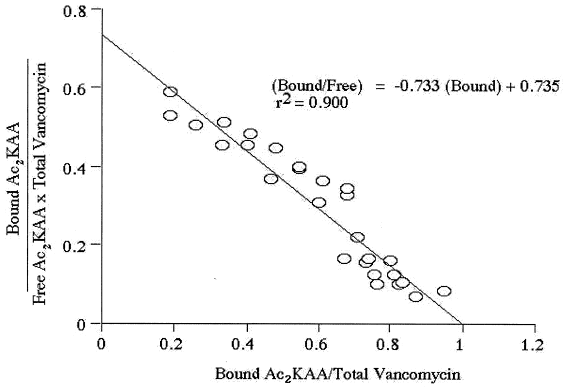

Fig. 9. Scatchard plot obtained by monitoring ESI-MS for the intensities of protonated Ac_2KAA during a titrimetric experiment involving Ac_2KAA and vancomycin. Reprinted with permission from Wiley, New York [72].

where K is also called the binding constant.

Several ESI-MS methods have been developed to estimate various $\log K$ values [71–79]. Most of the methods entail monitoring the intensities of both the free, unbound guest and the bound host–guest complex or the free, unbound host and the bound host–guest complex over a range of concentrations. This titrimetric data are used to construct a Scatchard-style plot. In this method, either the free host and host–guest complex or the free guest and host–guest complex must be ionic and within the mass range of the mass analyzer so that they can be detected mass spectrometrically. The binding or dissociation constants for oligonucleotide-serum albumin complexes [71], vancomycin/peptide complexes and ristocetin/peptide complexes [72,75,76], protein/phosphopetide complexes [73], and aminoglycoside/RNA models [77] have been measured by using this method. An example is shown in Fig. 9 for the binding of vancomycin to *N*,*N*-diacetyl-$_L$-Lys-$_D$-Ala-$_D$-Ala-$_D$-Ala (Ac_2KAA) [72]. The vancomycin was titrated with an increasing concentration of Ac_2KAA, and the ratio of the bound Ac_2KAA to the product of the free Ac_2KAA and total vancomycin, measured based on mass spectral intensities, was plotted against the ratio of the bound Ac_2KAA to the total vancomycin. Fig. 9 shows the resulting Scatchard plot, in which the binding constant was calculated as 7.33×10^5 L mol^{-1} (based on the slope) [72], in good agreement with the binding constant obtained by using a conventional method.

Another method used to determine binding constants involved monitoring the intensities of the free and bound host and guest species in solution, all at equimolar initial concentrations. The ratios of the resulting host–guest complexes were used to determine the binding constants of each host–guest complex. This method, which assumes that the ESI efficiencies of the various host–guest complexes and unbound species are similar, has been used to determine the binding constants of vancomycin antibiotic/peptide complexes [72]. A related method developed by Liu and co-workers incorporated the use of an internal standard (i.e. a reference host–guest complex) to account for the differences in ESI efficiencies of the host–guest complexes of interest [74]. This method was used to estimate the binding constants of a series of lariat ether/alkali metal complexes.

The most recent method involves a competitive equilibrium experiment in which the intensity of a reference host–guest complex is monitored after the addition of a second host or guest of interest to perturb the equilibrium distribution of complexes [79]. The change in intensity of the reference host–guest complex relates to its change in concentration in the solution, thus reflecting the binding constant of the second host or guest. This method may be used to determine binding constants of complexes that are neutral or that have mass-to-charge ratios higher than the range of the mass spectrometer because only the intensity of the reference host–guest complex is recorded. Because only one complex is monitored, differences in ESI efficiencies between the host–guest complexes or bound and free species do not influence the accuracies of the measured binding constants.

4. Conclusions

Over the past decade, mass spectrometry has allowed the first studies of the intrinsic aspects of host–guest chemistry in a solvent-free environment. Binding affinities can be probed by examination of

ion-molecule reactions or by collisional activated dissociation of 2:1 host:guest complexes. Both size-selectivity and electronic effects influence the formation, reactions and stabilities of host–guest complexes in the gas phase. More recently, electrospray ionization mass spectrometry has become a promising new way to monitor molecular recognition in solution. The electrospray ionization process is gentle enough to transfer noncovalent complexes to the gas phase for analysis, and the intensities of ions observed in the mass spectra can be correlated with the equilibrium distribution of complexes existing in the solution. Because of its low sample consumption, access to structural information, and compatibility with a wide range of solvents, ESI-MS will find increasing applications for solving more complex problems in molecular recognition over the coming decades.

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