Electrospray mass spectrometry and supramolecular complexes: quantifying the metal ion binding properties of cholic acid derivatives

Paul A. Brady and Jeremy K. M. Sanders*

Cambridge Centre for Molecular Recognition, University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW

An electrospray mass spectrometry protocol has been developed for the rapid screening of metal ion binding affinities, and it has been applied to the study of the alkali metal ion binding characteristics of neutral monomeric and macrocyclic steroid derivatives. It is shown that experiments involving competition between a range of metal ions and a single host can yield useful information on relative binding affinities; these results correlate well with synthetic templating results in solution. The reverse experiment, in which several hosts compete for the same ion, does not give reliable results (*a*) because unbound hosts remain uncharged and are not detected in the spectrometer and (*b*) because different hosts display radically different inherent detectabilities.

Electrospray ionisation has become a dominant technique in mass spectrometry in the last half decade. As ions are generated directly from aqueous solution with only mild heating, electrospray ionisation mass spectrometry (ESI-MS) is suitable for the study of intermolecular processes and as a detection system for liquid chromatography.[1] The conditions can approach physiological, so the technique is ideal for the study of proteins: the mass spectrometry of polypeptides with molecular weights of up to 100 kDa is now almost routine.[2,3] Recent examples include studies of enzyme–substrate, antibody–antigen, drug–receptor and substrate–receptor[4–7] interactions. ESI-MS has also found application in the field of supramolecular chemistry for the investigation of synthetic host–guest[8,9] and host–metal ion systems.[10–13] In many cases, qualitative agreement is observed between binding characteristics found using ESI-MS and by more conventional solution phase binding studies. As ESI-MS requires only very little material and allows rapid analysis, it has the potential to be a very powerful technique for the analysis of binding interactions.

However, the method is associated with many variables, the importance of which are still unclear. Recently, many results have been accepted without the background processes being fully understood. For example, aggregate ions are seen in small quantities in many ESI spectra, particularly at high analyte concentrations. The observation of expected complexes is quite frequently accompanied by stoichiometrically nonsensical adducts, which must surely limit the conclusions that can be drawn from such experiments. The environment in which a molecule finds itself when being analysed in the mass spectrometer is so different from that in solution that the mechanism by which ions are generated deserves careful scrutiny. As the generation of ions involves only brief and minimal heating, the preservation of intermolecular interactions in the spectrometer is not in itself an implausible suggestion; however, it is not clear that this preservation fits current models of the ESI ionisation process. As the generated ions are stripped of solvent by electrostatic forces and evaporation processes, highly desolvated ions are generated, but it is difficult to see why this process should so readily remove non-covalently associated solvent molecules whilst leaving other non-covalent associations intact.

* E-mail: jkms@cam.ac.uk

Smith *et al.*'s studies have gone some way towards clarifying the conditions under which specific binding can be characterised by ESI-MS.[1,14] In particular, it is proposed that structurally modified receptors should be shown to fail to aggregate in order to demonstrate that a specific binding interaction is responsible for the formation of an adduct. In the area of metal ion binding, Leize *et al.* have found a direct correlation between solvation energy and ESI-MS response for the alkali metal ions in aqueous solution.[15] Factors such as solvation energy must therefore be taken into consideration when quantitative information is extracted from mass spectra. For instance, the association of two enzyme subunits generates a product that differs in molecular weight and surface characteristics from the separate halves, so the mass spectral response cannot be assumed to be the same for each component. Such discrepancies have been found to be less marked in metal ion binding experiments;[14] here the ion makes only a minor contribution to the total mass of the complex and, if effectively bound, has only little exposure to the solvent. A molecular mechanics study of 18-crown-6–metal ion complexes showed that the solvation energy of the complex was virtually independent of the metal ion present.[16] As good agreement was observed between binding affinities deduced from mass spectral data and binding constants measured by other methods in this and other cases,[17] the assumption that complexes of a given host with a variety of ions give the same mass spectral response intensity appears to be justified. This remains to be proven experimentally.

Following our observations in thermodynamic cyclisation reactions carried out in the presence of metal ions as templates,[18] we wished to screen the metal ion binding ability of the steroid derivatives concerned. Initial trial ESI mass spectra showed that in all cases, with no added metal salts, the main ion observed for the steroid monomers and oligomers was the $(M + Na)^+$ ion. This is the result of contamination with sodium ions from the solvent, the glass bottles and tubes employed and probably also from the spectrometer chamber itself. Some other ions were also occasionally seen [*e.g.* $(M + K)^+$, $(M + NH_4)^+$] but an $(M + H)^+$ ion was never observed. Before engaging in a study of the metal ion binding characteristics of these molecules, it was necessary to clarify some of the ESI-MS variables and to develop a protocol that would allow meaningful data to be obtained from mass spectra in a reliable fashion.

In this paper we show that in metal ion binding studies, a

competition experiment in which several hosts compete for the same ion does not deliver meaningful information. However, reliable *relative* binding data can be obtained if several ions compete for a given host, and we show that this approach can deliver a large amount of useful data in a relatively short time. This paper does not address the more fundamental issues of the exact nature of the ionisation process and how that affects the quantification of solution phase behaviour using ESI-MS.

Results and Discussion

Initial explorations

The first question to be addressed was whether the intensity of the mass spectral peak was directly proportional to the amount (or the concentration) of material added for these molecules. To investigate this, samples at concentrations between 2 μM and 50 μM were injected into the spectrometer. The results for the MEM monomer **5** and the MEM trimer **5c** are shown graphically in Fig. 1(*a*) and 1(*b*). Both sets of data show good linearity in the examined concentration range, but there is a considerable difference in the scales of the two graphs: the response for the monomer **5**, $(M + Na)^+ = 623$,† is roughly 120 times more intense than the response for the trimer **5c**, $(M + Na)^+ = 1727$. It is not clear whether this difference is the result of variation in instrument sensitivity for the different mass ranges, or due to different quantities (ion current) of the ions being produced, perhaps due to radically different solvation energies.

Intermolecular binding experiments are complicated by the fact that the majority of ions and molecules that enter the spectrometer chamber do not reach the detector. In particular, in a metal ion binding study, any uncharged host molecule that has no metal ion attached is not observed at all. Thus, in a competition experiment in which two molecules compete for the same ion, the strong binding of the metal ion by one of the hosts may cause a large adduct peak to be detected, but may not reduce the free metal ion concentration sufficiently to prevent the simultaneous observation of a large adduct peak for a weaker binding host. This can even be the case when there is a substoichiometric quantity of metal ions, as unbound hosts remain uncharged and are not detected. Thus, the intensity of the peak will only show how much better the host molecule binds the ion than it binds H^+ or solvent, not how much better it binds the ion than the other molecule does. Corrections for this, considering the relative fractions of the total ion current, are not possible as the sensitivity varies with mass and from molecule to molecule as shown above. Thus a competition experiment in which several molecules compete for the same ion cannot yield meaningful data.

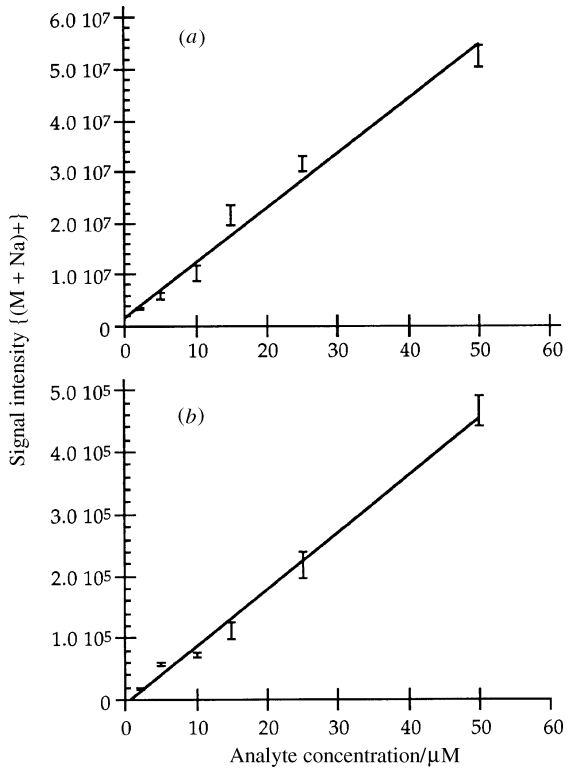


Fig. 1 Dependence of the mass spectral signal intensity on the concentration of analyte: (*a*) MEM monomer **5** and (*b*) MEM trimer **5c**

† The convention adopted throughout this paper is that $(M + X)^+$ represents the adduct ion, $\{(M + X)^+\}$ denotes the intensity of the peak for the $(M + X)^+$ adduct in the mass spectrometer and $[(M + X)^+]$ denotes the concentration of the adduct.

The next stage was to assess the metal ion binding response. As described above, the dominant peak in all our spectra is always the $(M + Na)^+$ peak, but initial experiments showed that it was possible to 'compete away' the sodium by addition of a second metal ion. When Li^+ was added to a solution of MEM trimer **5c**, two peaks were observed at $m/z = 1711$, $(M + Li)^+$, and 1727, $(M + Na)^+$. The linearity was investigated by means of titration experiments. A solution of MEM trimer **5c** (at 25 μM concentration) was injected into the spectrometer in the presence of increasing amounts of Li^+, giving the results shown in Fig. 2. Analogous experiments with addition of potassium and cesium ions were also carried out (results not shown). In order to analyse these results, it is necessary to consider the equilibrium between the species observable in the mass spectral experiment:

$$(M + Na)^+ + Li^+ \overset{K_{eq}}{\rightleftharpoons} (M + Li)^+ + Na^+ \qquad (1)$$

where K_{eq} is given by:

$$K_{eq} = \frac{[(3mer + Li)^+][Na^+]}{[(3mer + Na)^+][Li^+]} \qquad (2)$$

Before acquiring the mass spectrum, the solutions were allowed to stand for 15 min to ensure that they had reached equilibrium. Assuming that the peak intensities $\{(3mer + Li)^+\}$ and $\{(3mer + Na)^+\}$ are proportional to the concentrations of the ions in solution $[(3mer + Li)^+]$ and $[(3mer + Na)^+]$, a plot of $\{(3mer + Li)^+\}/\{(3mer + Na)^+\}$ against $[Li^+]$ will have a slope of $K_{eq}/[Na^+]$. If $[Na^+]$ is the same in each experiment the relative binding affinities of Li^+, K^+ and Cs^+ can be calculated. They are shown in Table 1. During the mass spectral experiment, all the ions become desolvated and the droplets of solvent in which they each find themselves become ever smaller. As this occurs, the concentrations of all the components increase, but as the analysis being carried out involves only the *ratios* of the ions in solution and the *ratios* of the ions observed, this concentration change does

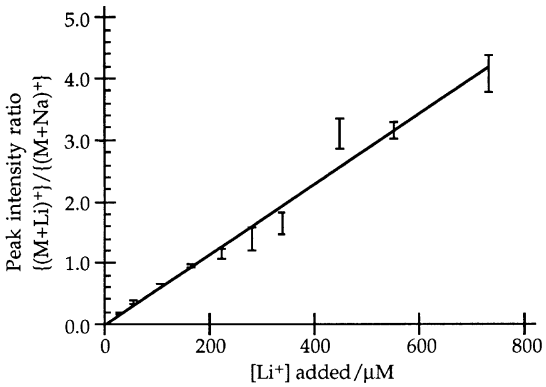


Fig. 2 Dependence of the ratio of $\{(M + Li)^+\}/\{(M + Na)^+\}$ mass spectral peak intensities on added $[Li^+]$

Table 1 The relative binding affinities of Li^+, Na^+, K^+ and Cs^+ to the MEM trimer **5c** and the background concentration of Na^+ [a]

Method	Li^+	Na^+	K^+	Cs^+
Slope K_{eq} *vs.* $[Na^+]/\mu M^{-1}$	$5.7\ (\pm 0.3) \times 10^{-3}$	—	$15.1\ (\pm 0.8) \times 10^{-3}$	$12.0\ (\pm 0.5) \times 10^{-3}$
$x/\mu M$	155 ± 16	—	162 ± 12	138 ± 20
Backtitration K_{eq}	0.91 ± 0.08	1.0	2.00 ± 0.19	1.72 ± 0.25
Single metal titration K_{eq}	0.86 ± 0.05	1.0	2.30 ± 0.12	1.82 ± 0.07
Simultaneous titration K_{eq}	1.02 ± 0.15	1.0	2.34 ± 0.14	1.74 ± 0.19

[a] Each row of values in the table was obtained by a different method; for details of the methods used, the reader is referred to the text.

not affect the calculation. These are competition experiments and as such they give rise to *relative* binding data. No attempts have been made to calculate absolute binding constants in this way.

The differences in the abilities of the different ions to compete away the sodium ions can be rationalised by their having different complexation affinities for the trimer, but could also be the result of different solvation energies of the different complexes, as discussed above. However, as the host molecule is the same in each case and is far larger than the metal ions, the solvation energies can reasonably be expected to be very similar. In any case, as solvation energies are lowest for large ions, peaks due to $(M + Cs)^+$ should be enhanced, as shown by Leize *et al.*[15] all other things being equal. Thus, the better competition ability of potassium can only be explained by a more favourable binding interaction.

In order to obtain more useful binding data from these experiments, it was necessary to establish the background concentration of sodium present during the analyses. By flushing the line with a concentrated solution of 18-crown-6 it was possible to reduce the level of sodium significantly, but a residual amount always remained. The level remained constant after further flushing and after several hours of normal spectrometer use; this level was thus considered a 'standard'. In order to calculate the concentration of sodium ions present, the following analysis was performed.

Considering the equilibrium previously described in eqn. (1) and the equilibrium constant in eqn. (2), addition of an excess of Li^+ will lead to the majority of ions produced being $(3mer + Li)^+$. If the distribution observed is a true equilibrium, then addition of extra Na^+ should increase the amount of $(3mer + Na)^+$ again. The amount of extra Na^+ added will be known, so the term for $[Na^+]$ can be replaced by

$$[Na^+] = N + x \tag{3}$$

where N is the known amount of $[Na^+]$ added and x is the background $[Na^+]$. Eqn. (2) can be rewritten as:

$$K_{eq} = \frac{[(3mer + Li)^+](N + x)}{[(3mer + Na)^+][Li^+]} \tag{4}$$

and an expression for N is obtained:

$$N = K_{eq} \frac{[(3mer + Na)^+]}{[(3mer + Li)^+]} \cdot [Li^+] - x \tag{5}$$

So a plot of N against $(\{(3mer + Na)^+\}/\{(3mer + Li)^+\}) \cdot [Li^+]$ will have a *slope* of K_{eq} and a y axis intercept of x. These experiments were carried out for lithium, potassium and cesium ions. The results of line fitting analyses to obtain values of x (the background sodium concentration) and K_{eq} are shown in Table 1. The graphical result obtained in the lithium case is shown in Fig. 3. The three separate experiments were in good agreement with both each other and previous titrations and give a background $[Na^+]$ of 152 ± 8 μM. With this information, it was possible to add sodium ion binding to the data in Table 1, standardising with respect to the sodium binding strength ($Na^+ = 1.0$).

The titrations described above are very time consuming and the experimental effort would be cut by a significant amount if several experiments were carried out simultaneously. Thus, the MEM trimer **5c** was titrated against all three ions together. As the concentration of lithium, potassium and cesium ions is increased, the intensities of the $(M + Li)^+$, $(M + K)^+$ and $(M + Cs)^+$ peaks increase while the intensity of the $(M + Na)^+$ peak is reduced. The results of this multiple experiment are best analysed by considering the ratios of the ion peaks. These manipulated data are plotted in Fig. 4. In the same way as before, the slopes of the three lines represent the relative binding affinities. These were calculated and are shown in Table 1.

As Table 1 shows, the overall agreement between the techniques is extremely good, and so this simultaneous monitoring technique was adopted as a general method for the analysis of metal ion binding affinities.

Relative ion affinities of steroid monomers

As previously noted, the spectra of the steroids studied always showed $(M + Na)^+$ as the major peak, with no $(M + H)^+$

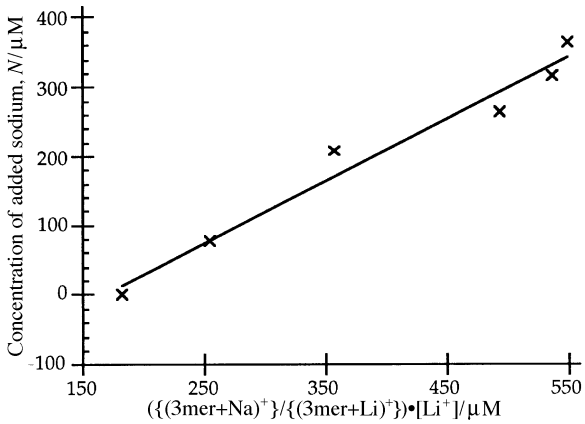


Fig. 3 Dependence of the known amount of sodium added against $(\{(3mer + Na)^+\}/\{(3mer + Li)^+\}) \cdot [Li^+]$

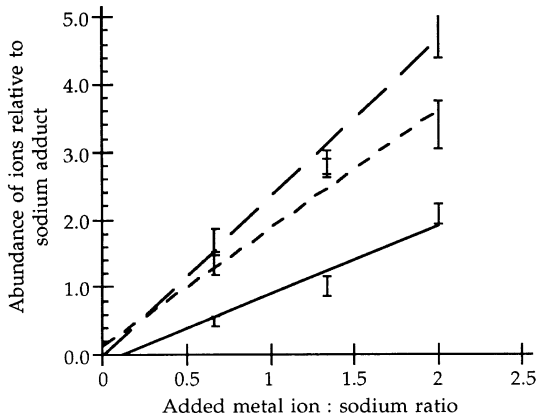
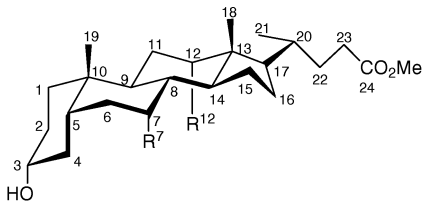
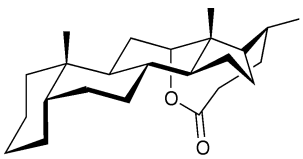


Fig. 4 Dependence of the abundance of the various adducts on the ratio of [added ions]/$[Na^+]$: (——) Li^+, (— —) K^+, (– – – –) Cs^+

being detected. This is also the case for the monomer molecules, which of course lack defined binding cavities. As a range of steroid derivatives had been synthesised, the effect of the different substituents on the ion binding profile was investigated. Thus, the series methyl cholate **1**, methyl deoxycholate **2** and methyl lithocholate **3** (bearing decreasing numbers of hydroxyl groups on the α face) were tested and the internal lactone **4**, bearing no hydroxyl groups, was also analysed. The selectivities of the cyclisation monomers **5** (MEM), **6** [di(*p*-methoxybenzyl)] and **7** (deoxy) were also determined. The relative binding affinities are shown graphically in Fig. 5. All of the monomeric hydroxylated bile acid methyl esters select almost exclusively Li^+ and Na^+ (>80%) from the mixture of ions. The ion binding profiles for these three molecules are essentially identical. In the light of the findings of Leize *et al.*[15] who showed that, all other things being equal, cesium ion adducts should be the most readily detected, this appears to be a significant result. The steroids seem to favour binding of the 'hardest' ion, that is the ion with the highest surface charge density. If the binding is due to a specific interaction, it probably involves the hydroxyl functions or the ester group as these are the only recognition sites present. Thus, Li^+–O appears to be the major binding interaction present.



	R^7	R^{12}
Methyl cholate **1**	OH	OH
Methyl deoxycholate **2**	H	OH
Methyl lithocholate **3**	H	H
MEM monomer **5**	$OCH_2OCH_2CH_2OMe$	OCH_2Ph
Di(*p*-methoxybenzyl) monomer **6**	O(*p*-OMe)CH_2Ph	O(*p*-OMe)CH_2Ph
Deoxy monomer **7**	H	O(*p*-Ph)CH_2Ph



Internal lactone **4**

The internal lactone **4**, which lacks hydroxyl functionality and possesses only the ester group, presents a slightly different profile from the bile acid methyl esters. It is able to bind potassium and cesium ions to a greater extent than the previously studied molecules. This result might be interpreted as implying that the hydroxyl groups favour Li^+ binding whilst the ester functionality has a broader, less specific affinity range. Additional experiments would be required to confirm this hypothesis.

By contrast, the three cyclisation monomers show significant affinity for potassium and cesium. The MEM monomer **5** selects potassium in preference to cesium, whilst the deoxy monomer **7** and the di(*p*-methoxybenzyl) monomer **6** bind both with roughly the same facility. The increased affinity for these ions over lithium [the $(M + Li)^+$ peak dominated for the unfunctionalised monomeric steroids] might arise from a π–cation interaction. Such interactions have previously been invoked to explain cation–calixarene binding observed by ESI-MS.[11] The deoxy and di(*p*-methoxybenzyl) monomers **7** and **6** are more effective in this respect, perhaps a consequence of their having extended, electron-rich π systems appended to the steroid skeleton. A π–cation interaction would be expected

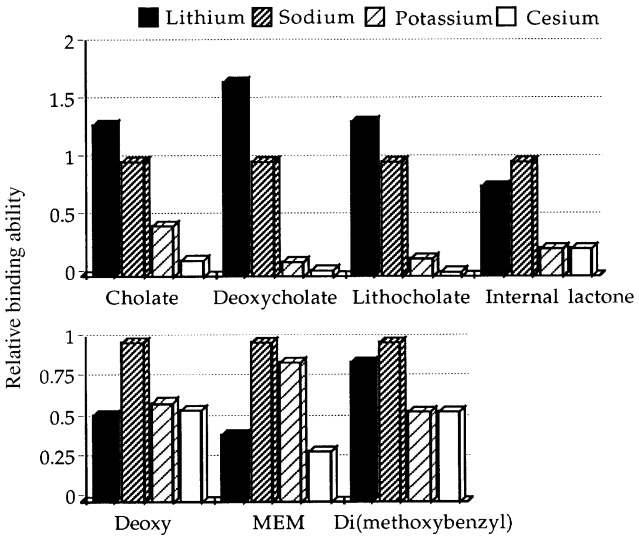


Fig. 5 Metal ion binding affinities of the steroid monomers to Li^+, K^+ and Cs^+ relative to $Na^+ = 1$. Binding abilities have been normalized with respect to Na^+ for each individual monomer or oligomer; they cannot be compared from one monomer to another

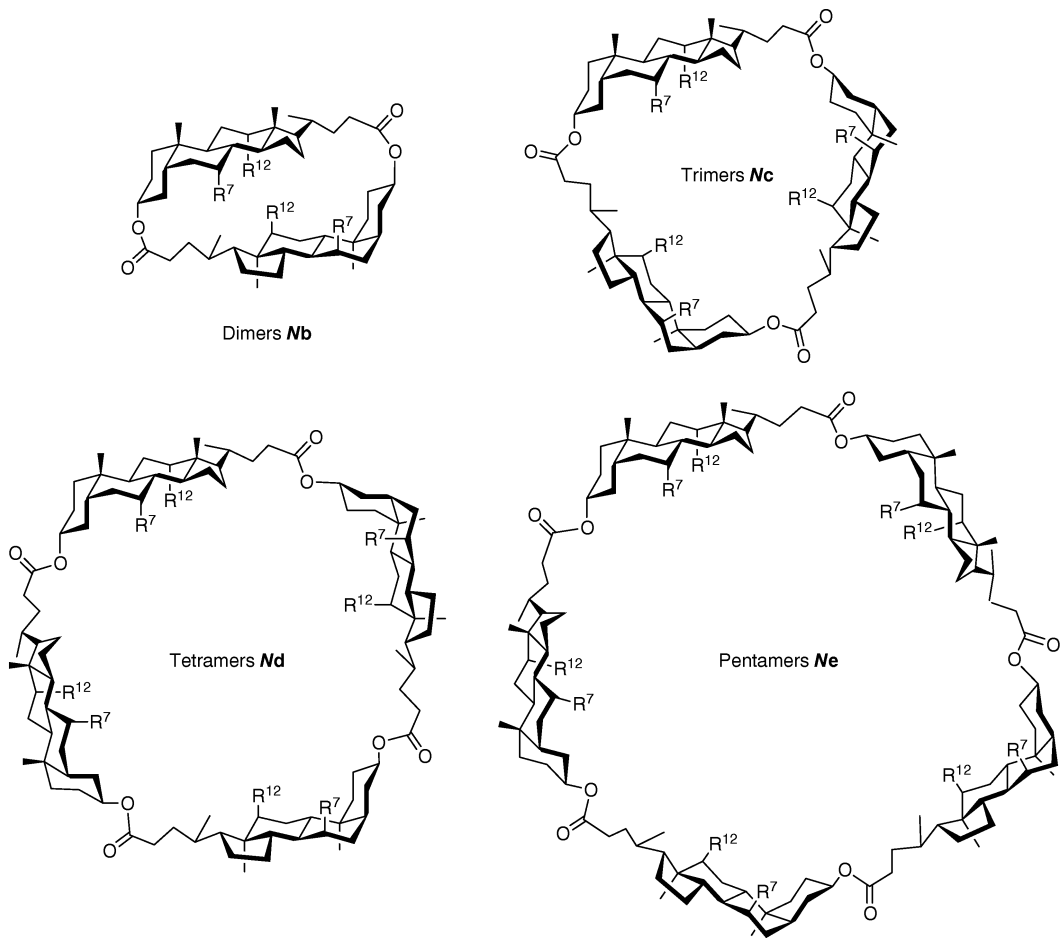
to be most effective for potassium binding[19] (as observed in the case of the MEM monomer) but other constraints, such as the di(*p*-methoxybenzyl) monomer's potential ability to form a sandwich complex by binding a metal ion between its two aromatic groups, might offset this. On the other hand, the presence of the polyether sidechain in the MEM monomer might impart extra potassium binding potential in addition to any π–cation effects.

These experiments show that it is possible to investigate relative binding affinities using ESI mass spectrometry. The variations observed suggest that some specific interactions are involved and that the ion binding profile is not merely statistical. Considering these results in the context of Smith *et al.*'s criteria for the presence of a specific interaction as detected by ESI-MS,[1,14] the complexes formed are of the correct stoichiometry, they are disrupted by changes in the interface conditions and they are affected by structural modifications in the substrate. The presence of an aromatic group seems to favour potassium and cesium binding.

Electrospray spectra of cyclic steroid oligomers

While the mass spectra of the monomeric steroids described above were very simple, consisting only of singly charged peaks (with no fragmentation products), which were easily assigned as $(M + Na)^+$ or other alkali metal adducts, the spectra of the cyclic oligomers were more complicated.

The only cyclic dimer investigated (cyclic deoxy cholate dimer **7b**) behaved in a similar way to the monomers, giving predominantly singly charged peaks. The trimers ***N*c** also gave mainly singly charged peaks as shown in Fig. 6. Small peaks (*ca.* 5% of the intensity of the singly charged ions) assignable as $(M + 2Na)^{2+}$ are usually also visible. In the MEM trimer titration experiments, the nature of the doubly charged peaks changed as the concentration of the other metal ion was increased. For lithium, initially $(M + Na + Li)^{2+}$ appears together with the $(M + 2Na)^{2+}$ ion. Upon further addition of lithium ions, the former peak begins to dominate and then, towards the end, $(M + 2Li)^{2+}$ becomes the major peak in this region of the spectrum. The intensities of these peaks are quite small (<5%) so they were not taken into account in the binding affinity measurements described above. However, analysis of their relative intensities was in good general agreement with two sequential binding interactions (using the same binding constant as calculated from the singly charged ion peaks). It is notable that whilst $(M + Na + Li)^{2+}$ and



$(M + 2Li)^{2+}$ were consistently visible in the lithium ion titration, in the potassium case only $(M + 2Na)^{2+}$ and $(M + Na + K)^{2+}$ were seen in that region of the spectrum. In the cesium experiment, no doubly charged species containing cesium were observed. When the concentration of cesium was increased and the $(M + Cs)^{+}$ ion became the dominant ion in the singly charged region of the spectrum, the effect in the doubly charged region was simply a reduction in size of the $(M + 2Na)^{2+}$ peak with no accompanying $(M + Na + Cs)^{2+}$ or $(M + 2Cs)^{2+}$ peaks being seen. The reduced affinity for a second metal ion strongly suggests that the ions are being bound inside the cavity: two lithium ions can easily be accommodated inside the MEM cyclic trimer, whilst two larger cesium or potassium ions cannot.

The cyclic tetramers ***N*d** appeared mainly as doubly charged species as shown in Fig. 7. A small amount of singly charged material was also visible in the spectrum. Unlike in the trimer case, all possible combinations of two metal ions bound to the molecules were represented in the spectrum. In titration experiments, the ion ratios were analysed as the sums of all peaks containing a given metal ion (with homo metal peaks counting twice). This treatment assumes independent binding of the two metals, but as no cooperative effects were visible, this appeared reasonable.

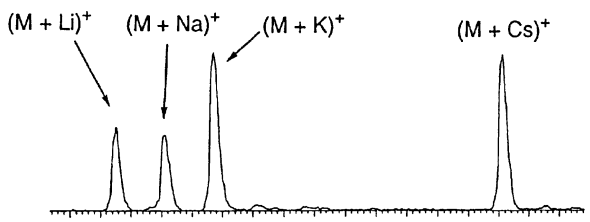


Fig. 6 Electrospray mass spectrum of MEM cyclic trimer **5c** in the presence of equimolar amounts of Li^{+}, Na^{+}, K^{+} and Cs^{+} ions

The cyclic pentamers also displayed mainly doubly charged peaks in their spectra, with no singly charged species detectable. No triply charged peaks were observed, so the cyclic pentamers were analysed in the same way as the cyclic tetramers.

Ion binding selectivity of steroid cyclic oligomers

The ion binding selectivities of the MEM macrocycles were investigated using the simultaneous titration method described. The results obtained, together with the outcome for the monomer, are shown in Fig. 8(*a*). For purposes of clarity, the peaks on the chart are normalised within each series. The trimer and pentamer display similar ion binding profiles, preferring potassium and cesium over sodium and lithium. These selectivities are similar to that of the monomer, but with a decreased affinity for sodium and an increased affinity for cesium. The remarkable result in this series is the selectivity

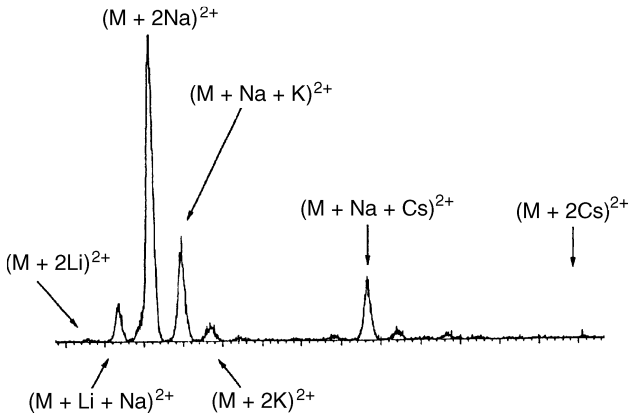


Fig. 7 Electrospray mass spectrum of MEM cyclic tetramer **5d** in the presence of equimolar amounts of Li^{+}, Na^{+}, K^{+} and Cs^{+} ions

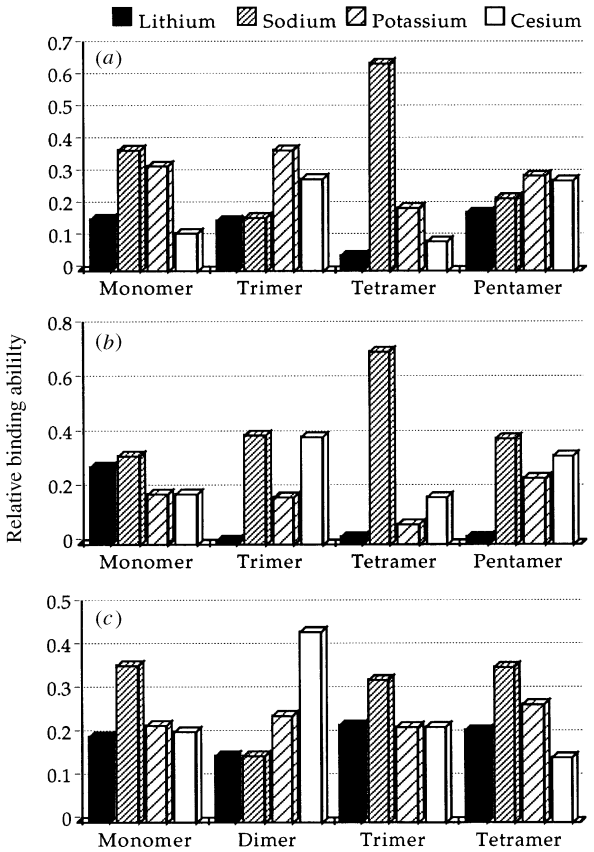


Fig. 8 Relative abilities of (*a*) the MEM series, (*b*) the *p*-methoxybenzyl series and (*c*) the deoxy series to bind to Li^+, Na^+, K^+ and Cs^+ ions. Binding abilities have been normalized for each individual monomer or oligomer; they cannot be compared from one monomer to another or between different oligomers derived from the same monomer

shown by the tetramer for sodium. It binds sodium three times as readily as potassium, and more selectively still when compared with the other ions. A possible explanation for this behaviour is that the tetramer's four polyether sidechains may define two binding cavities similar to 15-crown-5: two sodium ions could thus readily be accommodated in the cavity, each ion interacting with two chains. In the pentamer, the cavity is larger and the geometry is different, so it lacks this ability and therefore has a different ion binding profile.

Results for the di(*p*-methoxybenzyl) cyclic oligomers are presented in Fig. 8(*b*). The binding affinities displayed by this series of oligomers in many ways follow the trends observed for the MEM molecules. Again, the trimer and pentamer show similar ion binding profiles with an increased affinity for cesium relative to the monomer. However, unlike the MEM series, the lithium binding ability is virtually eliminated in these macrocycles whilst sodium complexation remains significant and always greater than potassium complexation. That sodium and cesium binding should both be favoured over potassium binding is curious. However, the behaviour of the tetramer is very similar to that previously observed, displaying remarkable selectivity for sodium binding. The explanation involving the polyether chains wrapping around central metal ions clearly cannot hold in this case, as only aromatic functional groups face into the cavity, so the origin of this binding preference is not clear. A possibility might be metal ion binding by the ester groups in the macrolactone linkages.

Results for the deoxy cyclic oligomers are shown in Fig. 8(*c*). In this series, the trimer and tetramer are seen to behave in a similar way to the monomer, with sodium being the preferred ion. Little discrimination is observed between the other metals. Unlike in the other two series, the deoxy cyclic oligomers retain significant lithium binding ability. The cyclic dimer is the only oligomer that shows a different profile. It possesses increased affinity for cesium, and a selectivity of greater than two for this metal over any of the other ions. Given the proximity of the two biphenyl groups enforced by the structure of the cyclic dimer, it is feasible that the two aromatic groups are orientated in a way that enables particularly favourable binding of cesium in a π-sandwich complex. The size of the ion might be critical for this interaction. This observation aside, the cyclic oligomers in the deoxy series have very similar ion binding profiles to the monomer. This might be a result of the fact that this building block only possesses one sidechain and therefore generates quite open and flexible cavities in the macrocycles. The sidechains in each oligomer have similar degrees of rotational freedom to the monomer and therefore might be expected to display similar ion binding behaviour.

Conclusions

Having established that host competition experiments cannot yield reliable data, we developed a method for the assay of metal ion binding using electrospray mass spectrometry. Initial experiments suggested that the simultaneous titration method was valid and good linear correlations were obtained, allowing investigation of the binding of alkali metal ions to steroidal hosts. The results obtained displayed a high degree of consistency and were reproducible.

A range of different behaviours was observed in metal binding by the monomeric steroid derivatives, suggesting that hydroxylated molecules bound lithium and sodium best, whilst appended aromatic groups favoured binding of potassium and cesium. Ion-binding by the macrocyclic derivatives was markedly different from that by the monomers. The presence of the cavity changes the binding profile significantly in some cases. For example, lithium binding, which is quite strong in the monomer, is abolished in the macrocycles for the di(*p*-methoxybenzyl) series. These differences constitute strong evidence that the binding profile results from specific interactions. To what extent binding data obtained from any mass spectral experiment represent a true reflection of solution state processes remains unclear.[20]

Irrespective of these limitations, significant binding selectivity was found in the cases of the MEM and di(*p*-methoxybenzyl) cyclic tetramers. Both display a strong preference for the binding of sodium. A similar, though slightly smaller effect is observed in the deoxy cyclic dimer, which showed a strong affinity for cesium ions. The modest ion binding selectivity observed is similar in magnitude to that in related molecules measured in solution by picrate extraction experiments by Bonar-Law and Sanders.[21] When these results are compared with the templating effects observed in the thermodynamically controlled synthesis of these molecules,[18] good agreement is found. The yield of cyclic tetramer was increased by the addition of sodium in the cases of the MEM and di(*p*-methoxybenzyl) monomers and these were found to bind sodium preferentially in the ESI-MS study. There is a similar match in the case of the deoxy dimer and cesium. We believe this supports our proposal that the changes in product distribution in the cyclisation reactions are the result of thermodynamic templating.

Experimental

The bile acid methyl esters **1**–**3** were available commercially, internal lactone **4** was synthesised as described by Schulze *et al.*[22] and the cholic acid derivatives **5** to **7** and their macrocyclic counterparts were available as described previously.[18] All of the experiments described were carried out using a VG BioQ ES mass spectrometer with a 1 : 1 (v : v) water–acetonitrile solvent system entering the chamber at a rate of 4 μL min^{-1}. The source temperature was 70 °C and the cone

voltage was set at 85 V. Sample solutions were in the concentration range 10–100 μM and made up in 10 : 45 : 45 (v : v : v) chloroform–acetonitrile–water. All spectra were analysed as the sum of at least 10 scans, each of 10 s duration. Metal ions were added as their iodide salts.

Acknowledgements

We thank the EPSRC and Rhone-Poulenc Rorer for financial support, Don Daley (RPR) for useful discussions on ESI-MS and Edward Ainscow (University of Cambridge) for advice on statistical analysis.

References

1 R. D. Smith, Q. Wu, Q. P. Lei and J. E. Bruce, *Chem. Soc. Rev.*, 1997, **26**, 191.
2 M. Przybylski and M. O. Glockner, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 806.
3 M. Vincenti, *J. Mass Spectrom.*, 1995, **30**, 925.
4 B. Ganem, Y-T. Li and J. D. Henion, *J. Am. Chem. Soc.*, 1991, **113**, 6294.
5 X. Cheng, R. J. Chen, E. Bruce, B. L. Schwartz, G. A. Anderson, S. A. Hofstadler, D. C. Gale, R. D. Smith, J. Gao, G. B. Sigal, M. Mammen and G. M. Whitesides, *J. Am. Chem. Soc.*, 1995, **117**, 8859.
6 M. J. Greig, H. Gaus, L. L. Cummins, H. Sasmor and R. H. Griffey, *J. Am. Chem. Soc.*, 1995, **117**, 10765.
7 M. C. Fitzgerald, I. Chernushevich, K. C. Standing, C. P. Whitman and S. B. H. Kent, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 6851.
8 J. M. Lehn, M. Mascal, A. DeCian and J. Fischer, *J. Chem. Soc., Chem. Commun.*, 1990, 479; J. A. Zerkowski, C. T. Seto and G. M. Whitesides, *J. Am. Chem. Soc.*, 1992, **114**, 5473.
9 K. C. Russell, E. Leize, A. van Dorsselaer and J. M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 209.
10 R. Colton, S. Mitchell and J. C. Traeger, *Inorg. Chim. Acta*, 1995, **231**, 87.
11 K. Wang, X. Han, R. W. Gross and G. M. Gokel, *J. Chem. Soc., Chem. Commun.*, 1995, 641.
12 F. Inokuchi, Y. Miyahara, T. Inazu and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1995, **35**, 1364.
13 L. J. Charbonnière, A. F. Williams, U. Frey, A. E. Merbach, P. Kamalaprija and O. Schaad, *J. Am. Chem. Soc.*, 1997, **119**, 2488.
14 R. D. Smith and K. J. Light-Wahl, *Biol. Mass. Spectrom.*, 1993, **22**, 493.
15 E. Leize, A. Jaffrezic and A. van Dorsselaer, *J. Mass Spectrom.*, 1996, **31**, 537.
16 P. Auffinger and G. Wipff, *J. Am. Chem. Soc.*, 1991, **271**, 163.
17 M. Goodall, P. M. Kelly, D. Parker, K. Gloe and H. Stephan, *J. Chem. Soc., Perkin Trans. 2*, 1997, 59.
18 P. A. Brady and J. K. M. Sanders, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3237.
19 D. A. Dougherty, *Science*, 1996, **271**, 163.
20 G. J. Langley, D. G. Hamilton and M. C. Grossel, *J. Chem. Soc., Perkin Trans. 2*, 1995, 929.
21 R. P. Bonar-Law and J. K. M. Sanders, *Tetrahedron Lett.*, 1992, **33**, 2071.
22 P. E. Schulze, A. Seeger and V. Illi, *Tetrahedron Lett.*, 1983, **37**, 2815.

Received in Montpellier, France, 19*th December* 1997;
Paper 8/00524I