Chiral recognition mass spectrometry: remarkable effects observed from the relative ion abundances of ternary diastereomeric complexes using electrospray ionization†

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The relative abundances of ternary diastereomeric complexes (composed each from a cinchonane-type chiral selector, a model chiral acid, and an alkali cation) are shown to change remarkably and fortuitously with variation in concentration and type of alkali metal using electrospray ionization-mass spectrometry and competitive binding analysis.

The use of electrospray ionization-mass spectrometry (ESI-MS) has been pursued as an alternate means for screening and assessing binding affinities and enantioselectivities of cinchonane-type chiral selector molecules for the separation of model chiral acids.[1] In the context of using mass spectrometry to measure solution phase associative equilibria, enantioselectivity (α_{MS}) is calculated from the relative abundances of diastereomeric ionic complexes observed in mass spectra. The results obtained thus far through ESI-MS screening and titration studies are promising; showing the preservation of pre-formed solution phase noncovalently-bound diastereomeric complexes and their detection in the gas phase with only minor distortion of equilibria. By focusing on well-characterized interaction partners, such as the chiral selector *tert*-butylcarbamoylquinine (tBuCQN; **1**) binding discriminatively the enantiomers of dinitrobenzoyl-leucine (DNB-Leu; **2**), a well-founded assessment of the validity of this approach is obtained. For example, trends in enantioselective performance of several cinchonane-type chiral selectors obtained *via* chromatographic measurements have been accurately reproduced *via* MS measurements.

Competitive binding experiments, where a chiral selector and both selectand enantiomers (with one enantiomer being isotopically labeled) are all present in the sample mixture, is a convenient strategy used to increase throughput and avoid run-to-run variability.[2,3] Fig. 1 depicts a mass spectrum obtained from an equimolar mixture of tBuCQN, DNB-(*R*)-Leu, and DNB-(*S*)-d_{10}-Leu. Here, α_{MS} is calculated from the ratio of relative abundances of the 1 : 1 diastereomeric complexes incorporating tBuCQN selector and each selectand.

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† Electronic supplementary information (ESI) available: Representative mass spectra and analysis of complex ion abundance/enantioselectivity data for added molar excess of XOAc (X = Li, K, Cs). See DOI: 10.1039/b512719j

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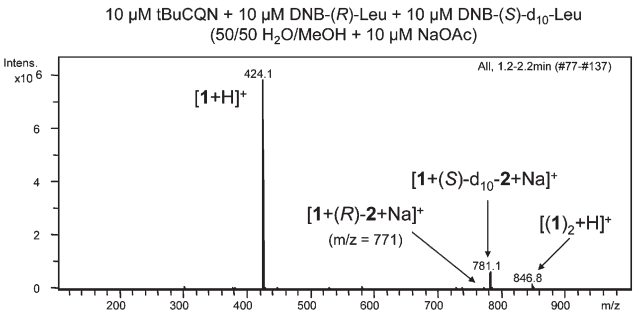

Fig. 1 Typical mass spectrum observed when applying a competitive binding strategy to evaluate α_{MS} (enantioselectivity) in a cinchonane-based chiral molecular recognition system. The dominant diastereomeric complexes between tBuCQN (**1**) and a quasiracemic mixture of isotopomeric enantiomers of DNB-Leu (**2**) appear as sodiated ions.

Control experiments designed to study the consistency of the acquired data yielded two intriguing issues which prompted further investigation. The first was the observation of a dominant sodiated ternary diastereomeric complex. Investigated here, the involvement of an alkali cation in the molecular-level chiral recognition mechanism, derived from conventional solution phase methods, of this system is a new phenomenon which has not been previously studied. The second was the presence of deuterium isotope effects which attenuate enantioselectivity measurements compared to those made without isotopically-labeled guest enantiomers. Deuterium isotope effects have been addressed in a separate communication.[4]

Although little thought was given to the sodiated 1 : 1 diastereomeric ionic complex during prior ESI-MS investigations, sodium ions in the form of 10 µM NaOAc were added to all analytical runs to equalize background sodium concentration and stabilize the ion signal. In this work, to more closely investigate the effect of this added sodium salt, a comprehensive study of salt type and concentration (given as molar excess relative to selector and selectand in the mixture) was performed. The result of this study was that an increase in the molar excess of alkali metal salt (across the range of concentrations tested) returned a significant increase in the measured α_{MS} (enantioselectivity; expressed as the quotient of the relative ion abundances of the ternary diastereomeric complexes). Fig. 2 shows how this effect was manifested with the increase of NaOAc concentration during successive analyses by ESI-MS. What is interesting is that whereas a substantial increase in ionic strength in an electrosprayed solution often suppresses ionization and/or host–guest association (exhibited in this system

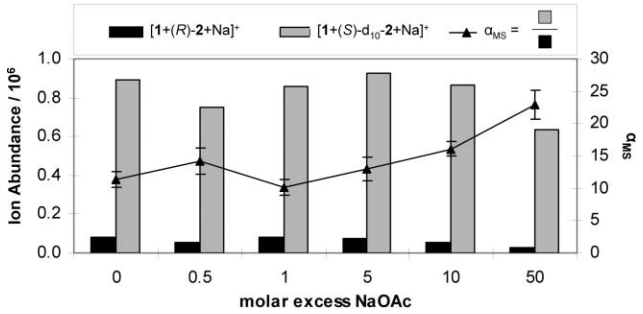

Fig. 2 Assessment of the increase in α_{MS} (enantioselectivity) for **1** binding isotopomeric quasienantiomers of **2** as a function of NaOAc molar excess (N = 3). Abundances of individual ternary diastereomeric ions are included to rationalize the observed trend. Analyte components are present at 10 μM each.

by the decrease in ion abundance of the lower affinity ionic complex ([**1** + (*R*)-**2** + Na]$^+$)), the higher affinity binding pair ([**1** + (*S*)-d_{10}-**2** + Na]$^+$) seems to better resist this effect.[5]

Following experiments using NaOAc, we also studied the effect of other salts in a similar manner (for representative mass spectra, see the Supplementary Information). Fig. 3, where the results obtained when different alkali metal acetate salts (XOAc, where X = Li, K, Na) were employed, emulates the results shown in Fig. 2. Studies were also performed using CsOAc, however the data are not incorporated into Fig. 3 because of large variations in some of the data points (α_{MS} = 30 ± 10 at 50-fold molar excess CsOAc), due to the instabilities of some ion abundances. A consistent increase in measured α_{MS} is observed. All measurements were based on the 1 : 1 diastereomeric ionic complex corresponding to the alkali metal cation employed (*e.g.*, [**1** + **2** + K]$^+$, when KOAc was added to the solution mixture). Behavior of the individual complex ion abundances also reflects similar stabilization/destabilization effects to those observed with NaOAc. Individual figures displaying these observations for XOAc are given in the Supplementary Information. For all alkali acetate salts investigated, the result is a remarkable enhancement in measured α_{MS} values with increasing molar excess of salt in the mixture.

To investigate possible counterion effects, chloride salts of sodium and potassium were evaluated in an identical manner to the acetate salts. The data revealed a similar behavior to that

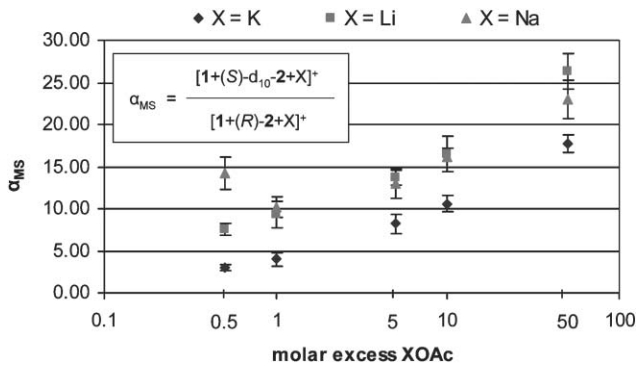

Fig. 3 Increase in α_{MS} (enantioselectivity) measured as a function of added (molar excess) alkali metal acetate salt for **1** binding isotopomeric quasienantiomers of **2** in an ESI-MS based competitive binding set-up (N = 3).

described above (data not shown). From these experiments, the counterion appears to have little effect on ion abundance and measured α_{MS} in this system. Acetic acid, ammonium acetate, and tetramethylammonium acetate additives were also investigated. For these solution modifiers, 1 : 1 complexes incorporating the cationic part of these salts (H^+, NH_4^+, and $N(CH_3)_4^+$, respectively) were not observed, even when present in 100-fold excess to selector and selectand molecules. Instead, normal ion suppression (host–guest destabilization due to increased ionic strength) effects resulted in an overall decrease in ion abundance with increasing salt concentration.

Measurement of enantioselectivities (α_{MS}) based on diastereomeric ion abundances allows for a quick and easy method to *qualitatively* assess relative binding affinities. To extract *quantitative* information related to the correlation between equilibrium constants of an interaction in solution and that represented by observed gas phase ion abundances, one must employ more rigorous titration-based methods. In our previous work, we demonstrated the applicability of quantitative methods for these systems.[1] In the current work, we rely on the validity of the assumption that we are operating in a good (linear) working range. Regardless, future work will incorporate quantitative techniques to test this assumption; focusing on the deconvolution of contributions by: (1) the effect of increased ionic strength on ionization efficiency for the ternary analyte system; and (2) the effect of ionic strength and solvation environment affecting binding equilibria between the co-analytes in solution and during their transfer into the gas phase. Focus is placed here on the phenomenon of cation attachment, relying on established screening protocols to learn more about the effect of cation type and concentration on relative ion abundances.

A substantial compendium of previous work has focused on the elucidation of the molecular-level chiral recognition mechanism for this model system.[6,7] Based on the wealth of mechanistic investigations, it is known that binding and enantioselectivity in this model system is achieved through an assisted, cooperative arrangement of intermolecular forces. Fig. 4 summarizes the contributing forces relative to the structure of the associates. Bimolecular association is driven by a hydrogen-bond-supported coulombic attraction between the quaternary ammonium group on the selector and the deprotonated carboxylate on the selectand. Additional hydrogen-bonding, π–π, and van der Waals forces then reinforce the stereoselective interaction, with one selectand binding the selector to a significantly greater degree than the other

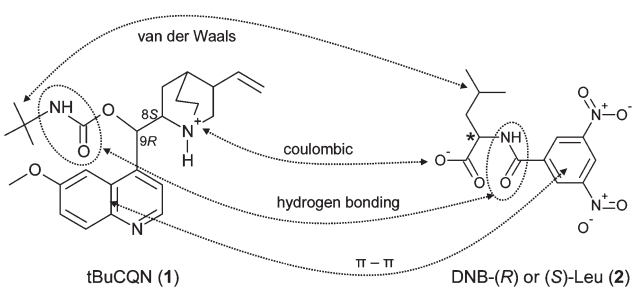

Fig. 4 Map of noncovalent interactions between chiral selector tBuCQN and chiral selectand DNB-Leu which result in enantioselective binding for this system.

($\Delta\Delta G > 6.5$ kJ mol^{-1} has been shown depending on experimental conditions).

Considering the close contact of the selector and selectand (induced by the alignment of functional units afforded by the primary coulombic interaction) and the relative similarities in enantioselectivity measured by chromatographic and MS measurements, it is unlikely that a sodium cation (Pauling ionic radius = 95 pm), for example, has been inserted between the complementary binding units. Rather, it is probable that the cation is externally associated with the stacked π–π interaction sites (*i.e.*, the 6-methoxyquinoline ring of the selector and the dinitrobenzoyl (DNB) unit of the selectand); creating a tertiary cation–π–π system of unknown arrangement. It is plausible that the added cation shifts the equilibrium toward greater association for the higher affinity pair; possibly stabilizing the noncovalent complex through favorable binding with the stacked π system. By suppression (or destabilization) of one ionic diastereomeric complex and stabilization (or less destabilization) of the other, the increase in salt concentration results in a significant increase in measured α_{MS}.

Cation–π and π–π interactions in synthetic and biological systems are widespread. A few related reviews can be found in the literature.[8–10] Cation–π interactions are manifested through a balance of electrostatic, dispersion, and polarization forces. Consequently, they can be quite strong (in the gas phase potassium binds more strongly to a benzene molecule than it does to a water molecule ($\Delta\Delta G \sim 4.2$ kJ mol^{-1} in favor of benzene)).[8] Generally, for alkali metal ions, a binding order of $Li^+ > Na^+ > K^+ > Cs^+$ can be attributed to electrostatics. Variations in this order are induced mostly by the polarizability of the aromatic with which the cation interacts. Aryl π–π interactions are weaker in comparison, but are still prevalent in both designed molecular recognition schemes, as well as in complex biomolecule arrangements.[10] Attractive electrostatic interactions arise from the quadrupole moments of the aromatic rings; however questions still remain as to the contribution by dispersion and polarization forces.

The ranking of enantioselectivity enhancements as a function of alkali metal ion shown in Fig. 3 is intriguing. At low molar excess, the ranking $Na^+ > Li^+ > K^+$ is observed. At high molar excess, the ranking changes to $Li^+ > Na^+ > K^+$; apparently reflecting the electrostatic cation–π binding trend discussed previously. At low concentration, the trend is likely distorted by the inherent background sodium concentration, known to be present in similar concentration to the analytes in nearly all commercial solvents. The ranking at high molar excess is indicative of expected results when screening a series of cations for attachment to a given aromatic system – in this case, a stacked π–π arrangement. Additionally, the electrostatic component in binding is increased upon transfer into a solventless environment; further stabilizing the diastereomeric complex with the most harmonious alignment of enthalpically-driven intermolecular forces.

Studies addressing ternary systems involving multiple aromatic and cationic moieties are more difficult to find in the literature.[11–14] As is the case with our system, it is often difficult to extract conclusive information about a single interaction when a concert of intermolecular forces is responsible for an observed complex.[8] Additional experiments in general are needed to gain greater insight into the specific modes of noncovalent binding in this ternary system and to investigate the contribution of solvent and ionic strength to both electrospray ionization efficiency and shifts in binding equilibria. This will include: (a) evaluation of alkali metal binding to the cinchonane-based system using solution-phase chromatographic, spectroscopic, and calorimetric measurements; (b) molecular modeling and *ab initio* calculations to study geometries and energetics of multiple cation–π–π arrangements; (c) systematic selector and selectand structural/functional variation in solution phase and MS-based measurements; and (d) quantitative validation of enantioselectivity enhancements and affinities using MS and titration-based measurements.

Observation of ternary diastereomeric ionic complexes in cinchonane-based chiral selector–selectand association by ESI-MS provides a new system to study general and chiral molecular recognition. Cation–π and π–π interactions have been identified as important contributors to biological and synthetic interaction schemes of a diverse nature. This communication serves to identify another vehicle whereby these effects could be both beneficial in improving enantioselectivity and chiral molecular recognition measurements by MS, as well as in studying specific contributions by individual noncovalent forces. It is apparent from this and other recent work that a more comprehensive understanding of alkali metal binding effects would be beneficial.[15,16] Support for this work was provided by the Austrian Science Foundation (FWF Project #P15482).

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