Periodicity in collision-induced and remote-bond activation of alkali metal ions attached to polyether ionophores

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The collision-induced dissociation of lasalocid and monensin A bound to alkali metal cations has been investigated using electrospray MS/MS. The binding affinity for the metal cations, as measured with collision-induced dissociation, was found to depend on their ionic radius, decreasing with increasing radius. Monensin A was observed to have a greater binding affinity for alkali metal cations than lasalocid acid.

Introduction

The polyether ionophore antibiotics lasalocid and monensin A, along with salinomycin and narasin, are common in coccidiosis prevention for poultry and livestock, and are also applied to growth promotion for ruminant mammals. Recently, concerns have arisen regarding drug resistance in the parasite population, and increased human consumption of these ionophores. Speculation over these concerns[1,2] has led the European Commission to propose a 'phasing out' of coccidiostat additives.[3] Because of this, many studies have been undertaken to extract, identify, and monitor concentrations of the polyether ionophores both *in vivo* and *in vitro*.[4]

Prior to 1997, the primary technique for detection and characterization of ionophores was based around post column derivatization to give UV-absorbing chromophore complexes for high-performance liquid chromatography (HPLC),[5–7] or by the use of thin layer chromatography (TLC).[8] With the advent of tandem mass spectrometry, and LC-MS/MS, fragmentation patterns can now be used in conjunction with chromatography to give a more complete picture of the molecular composition of a chromatographic peak.[9,10] This had led to a significant amount of research in the field of low-energy collision induced dissociation (CID) spectra of the polyether ionophores. Both the free acid form $[Ionophore\text{-}H]^+$,[11] and the alkali metal adducts $[Ionophore\text{-}M]^+$ (M = Li, Na, K, Rb, Cs)[12,13] have been studied in-depth by CID. Detection and quantification of polyether ionophore antibiotics such as lasalocid and monensin A by LC-MS/MS is much simpler and more accurate than currently available solution phase techniques such as HPLC, UV-Vis spectroscopy, or electrochemistry.[14–17] With the MS/MS technique (commonly called CID), both parent ions and fragment ions can be compared to a library giving a greater level of assurance of the detection of a specific ionophore. Electrospray ionization (the most common source for LC-MS/MS) does not necessarily directly transfer the exact structural conformation from the aqueous to the gas phase, but determination of the gas phase chemistry is an important step toward understanding of the solution phase behaviour of a molecule.

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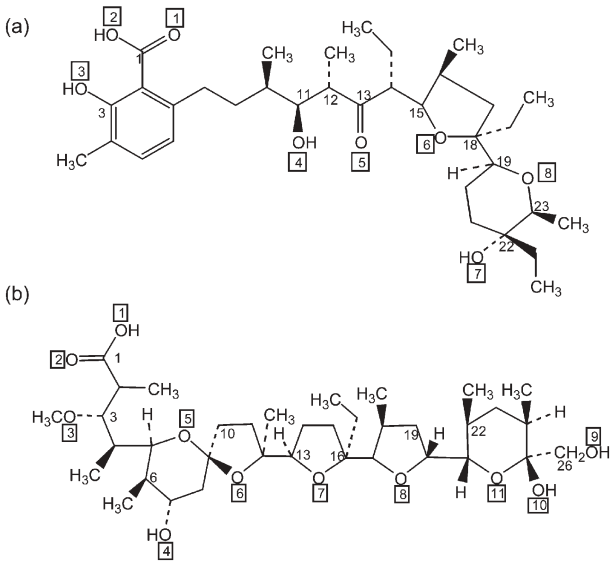
The basis of the previous research has been the determination of optimum methods for screening ionophores from important tissue substrates (liver, eggs, *etc*). A great deal of research has been conducted to determine solution phase binding constants.[18–22] However, no gas phase data has appeared in the literature at this point describing either metal affinity or complex stability. Understanding gas-phase interactions is an important step in the determination of the solution-phase behaviour of any biologically important molecule. Specific chemistry may be more easily probed in the gas phase because of the absence of solvent interactions.

The basic structure of the ionophores consists of a polyether backbone with a terminal carboxylic acid. This structural feature gives the ability to form non-ionic and ionic complexes with mono and divalent cations in solution. The complexation of alkali metal ions in solution occurs by a hydrogen bond between the terminal carboxylic acid and a hydroxyl group forming a cyclic binding site.[23] Both lasalocid and monensin A have been shown by NMR studies to undergo large conformational changes on complexation with alkali metals in the solution phase.[24–26] These studies have also shown the primary coordination sites of the alkali metal to the ligand in the solution phase which are similar to the solid phase crystal structure coordination sites as shown by X-ray studies.[27,28] It is this coordination which facilitates the transport of metal cations across biological membranes, and leads to the ionophores physiological and toxicological importance. The structure of lasalocid is shown in Scheme 1a. The lasalocid backbone is highly flexible, and very conformationally adaptable. Although similar in structure and mode of operation to lasalocid, monensin contains a much more rigid backbone, leading to less conformational movement. This is because the monensin A backbone contains fused ring structures. As a direct result, fragmentation of monensin A will be more likely to require ring opening. Because of the greater amount of rearrangement required to facilitate these ring openings, the fragments are expected to appear at higher collision energies. The structure of monensin A is shown as Scheme 1b. Monensin B is a slight structural variation on the A form where the ethyl group at carbon 16 (as labeled in Scheme 1b) is replaced by a methyl group. Due to the obvious similarities, only the A form has been studied at this time.



Scheme 1 (a) Lasalocid and (b) monensin A acids. Relevant carbon and oxygen atoms are labeled as *x* or *y* respectively.

Previous studies on the fragmentation patterns of both alkali metal lasalocid and monensin A acid cations have given an in-depth database of fragments that can be formed under CID conditions.[12,29,30]

In this paper we report the results of experimental investigations of the interaction of alkali metal cations with lasalocid and monensin A, two important ionophores, using electrospray ionization (ESI) tandem mass spectrometry (MS/MS). The principle focus of the research was the determination of the relative ability of the binding of alkali metal cations to an ionophore and the influence of the nature of the alkali cation on this bonding. Insight also was obtained on remote bond activation by an alkali metal cation due to the coordination mode of the metal. Remote bond activation by a metal cation (commonly leading to remote functionalization) has long been a tool of gas phase organometallic chemistry.[31,32] In such activation a metal cation activates a bond (usually C–C or C–H) not directly bound to itself, and facilitates or enhances the rate of an intramolecular rearrangement. Schwarz *et al.* have characterized many remote bond activation processes, generally with transition metals, in the gas phase.[33–35]

Experimental

Chemicals

Lasalocid, monensin A, HPLC grade methanol, formic acid and the alkali salts (NaI, KI, RbI, CsI, and LiCl) were obtained from Sigma Aldrich (Mississauga, ON, Canada).

Preparation of solutions

Lasalocid was dissolved in methanol at a concentration of 1.63 μmol L^{-1} and the appropriate alkali salt was added to ten-fold molar excess. Monensin A samples were prepared in a similar fashion at a concentration of 1.45 μmol L^{-1}. Both ionophores were acidified with 0.1% (v/v) formic acid.

Electrospray mass spectrometry and MS/MS

Electrospray data was acquired using an API 2000 (MDS-SCIEX, Concord, ON, Canada) triple quadrupole ($Q_1q_2Q_3$) mass spectrometer in the positive ion mode, equipped with a "TurboIonSpray" ion source. Experiments were performed with an ion spray voltage of 5500 V, a ring-electrode potential of 300 V, and a 70 V potential difference between the orifice and the skimmer. N_2 was used as a curtain gas at a flow rate of 10 (arbitrary instrument units), and air was used as a nebulizer gas at 20 (arbitrary instrument units). Samples were directly infused into the electrospray source at 3 μL min^{-1}.

MS/MS was carried out in the product ion and multiple reaction monitoring (MRM) modes with N_2 as collision gas ($p(N_2)$ = 2 [arb. units]). The collision offset voltage (the potential difference between the quadrupole entrance lens (q_0) and the collision cell quadrupole (q_2)), which gives the laboratory frame collision energy was investigated between 5 V and 130 V. Product ion spectra were then obtained by scanning Q_3 over the mass range *m*/*z* 5–1500. The inter-quadrupole lens potentials and the float potential of the resolving quadrupole Q_3 were linked to the q_2 potential, to maintain proper transmission through Q_3.

The onset energy of a particular fragment was determined by obtaining the steepest slope from the relative abundance plot of a particular ion, and extrapolating to the *x*-axis, as shown in Scheme 2. The error on these onset energies is determined as one standard deviation from the mean onset energy value calculated across the course of four repeated experiments.

Results and discussion

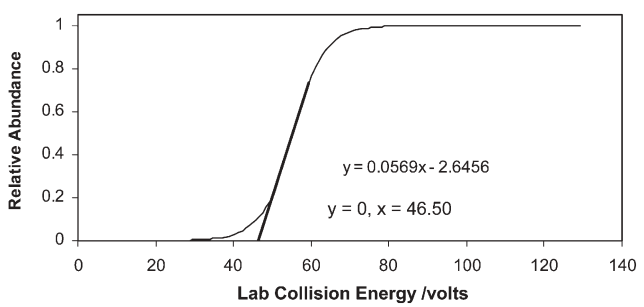
1. Dissociation of alkali metallated lasalocid acid cations

Aside from the elimination of water, alkali metallated lasalocid acid cations have been shown to dissociate in the following pattern.

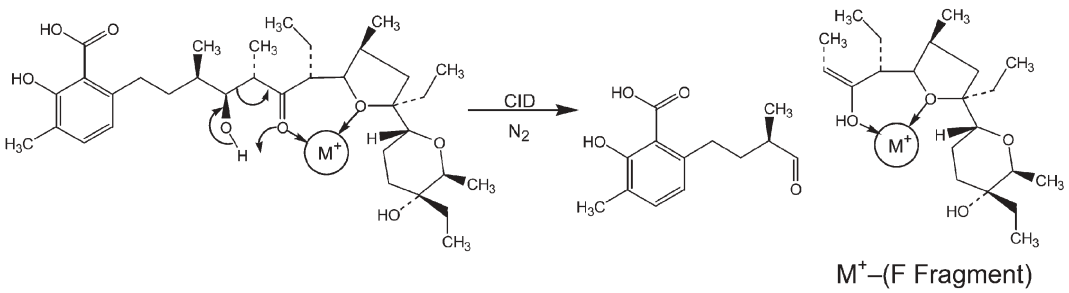
$$[\text{Las-M}]^+ \rightarrow [\text{Las-M}-236\text{amu}]^+ \rightarrow M^+$$

In 2002, Lopes *et al.*[29] presented a mechanism for the formation of the F fragment (where F = $[\text{Las-M}-236\text{amu}]^+$) in which a six membered intermediate facilitates an E_i type β-elimination.[36] This mechanism is shown below as Scheme 3.

This mechanism is promoted by the alkali metal withdrawing electron density from the carbonyl group due to the



Scheme 2 An example of the determination of an onset energy using the "steepest-slope method".



Scheme 3 Formation of [Las-M−236amu]$^+$ by β-elimination (E_i type) as proposed by Lopes *et al.*[29]

ion–dipole interaction. It is thereby assumed that an alkali metal ion with high charge density (such as Li^+) will enable this rearrangement to occur at a much lower CID energy than a low charge density ion (*i.e.* Cs^+).

The onset energies of two fragments of alkali metallated lasalocid cations ([Las-M]$^+$) were measured by MRM, *i.e.* the alkali metal dependant onset energy of [Las-M−236amu]$^+$ (the F fragment) and the onset energy of the free metal ion M^+. Results are presented in Table 1 and in Fig. 1(a) and (b) .

2. Dissociation of alkali metallated monensin A cations

Fig. 2 gives the experimental M^+ onset from alkali metallated monensin A ([Mon-M]$^+$) species.

Similar to lasalocid, monensin A has been studied extensively in the past. Lopes *et al.*[30] have proposed a series of fragmentation mechanisms explaining the observed MS/MS product ions for both the A and B forms of sodiated monensin. An example MS/MS spectrum for [Mon+Li]$^+$ at 90 V collision energy is given as Fig. 3, where both H_2O neutral losses from the parent ion and smaller fragment ions can be observed. [Mon+Li]$^+$ was found to fragment by a similar pattern as [Mon+Na]$^+$, but above 90 V (lab) collision energy (approximate onset energy of Na^+ in [Mon-Na]$^+$ case), fragments were observed that were not detected in the [Mon+Na]$^+$ case (*e.g.* at *m*/*z* 389, 306, 175, 106). Unfortunately, due to the limited instrument resolution at high collision energies, no structures could be assigned to these fragments. [Mon+K]$^+$ did only yield a single water loss (*m*/*z* 691.8) from the parent ion (*m*/*z* 709.8), and the free metal ion (K^+), while [Mon+Rb]$^+$ and [Mon+Cs]$^+$ gave only their respective free metal ions in the MS/MS spectrum. This lack of fragmentation by the K^+, Rb^+ and Cs^+ species can be accredited to the functional groups of monensin A. The main backbone of monensin A, unlike lasalocid, does not contain groups that are easily eliminated (*i.e.* β-hydroxyl groups). Therefore, collision energies much in excess of the Mon-M^+ bond dissociation energy (which will be comparable to the onset energy of the M^+ fragment) are required to fragment the backbone. As the charge will always be carried primarily on the alkali metal, no fragmentation of the monensin A backbone is observed for the larger [Mon+M]$^+$ species.

Table 1 Experimental data obtained from MRM scans of alkali metallated lasalocid acid cations parent species

Parent species (*m*/*z*)	M^+ ionic radius/Å	Onset energy of [F frag-M]$^+$/V	Onset energy of M^+/V
[Las-Li]$^+$ (597.8)	0.68	25.45 (3.18)	110.32 (17.65)[a]
[Las-Na]$^+$ (613.8)	0.97	34.47 (1.12)	78.80 (1.97)
[Las-K]$^+$ (629.8)	1.33	32.27 (1.30)[a]	46.50 (1.34)
[Las-Rb]$^+$ (675.8)	1.47	30.29 (4.69)[a]	39.37 (0.89)
[Las-Cs]$^+$ (723.8)	1.67	—[b]	29.04 (0.39)

[a] Note: Lower limit value. [b] No product ion observed. Error values in parentheses. Ionic radii taken from CRC Handbook.[37]

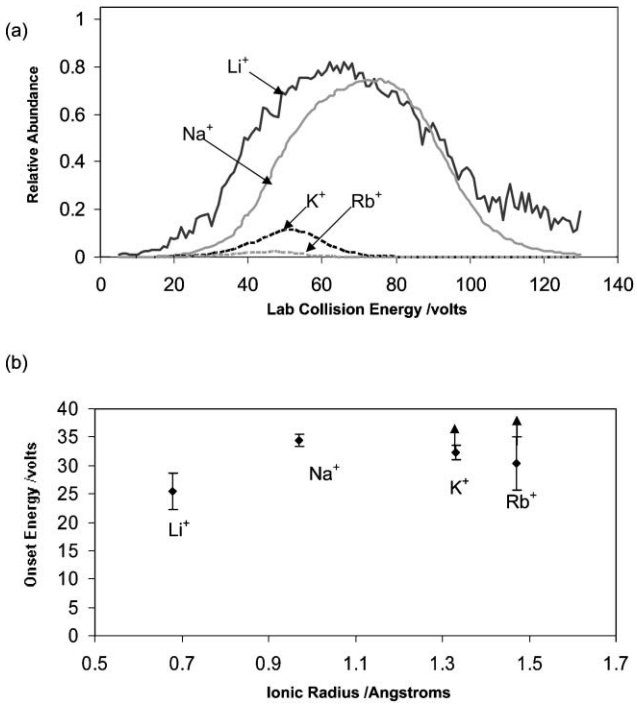


Fig. 1 Precursor ion [Las+M]$^+$ (a) Relative abundance of [Las+M−236amu]$^+$. (b) Onset energy of [Las+M−236amu]$^+$ down Group I. Error is defined as one standard deviation from mean calculated from four repeated experiments. Vertical arrows for K^+ and Rb^+ points indicate lower limit values. Note: [Las-Cs−236amu]$^+$ not observed. Ionic radii taken from CRC Handbook.[37]

3. Remote bond activation

The formation of the fragment [Las-M−236amu]$^+$ (the F-fragment) is an example of remote bond activation by an alkali metal. Li^+ has a very high charge density, and withdraws a large amount of electron density from the carbonyl group oxygen (O(5) Scheme 1a). This facilitates hydroxyl hydrogen (O(4) Scheme 1a) to migrate toward the carbonyl and yield a six-membered structure in the parent species (Scheme 4).

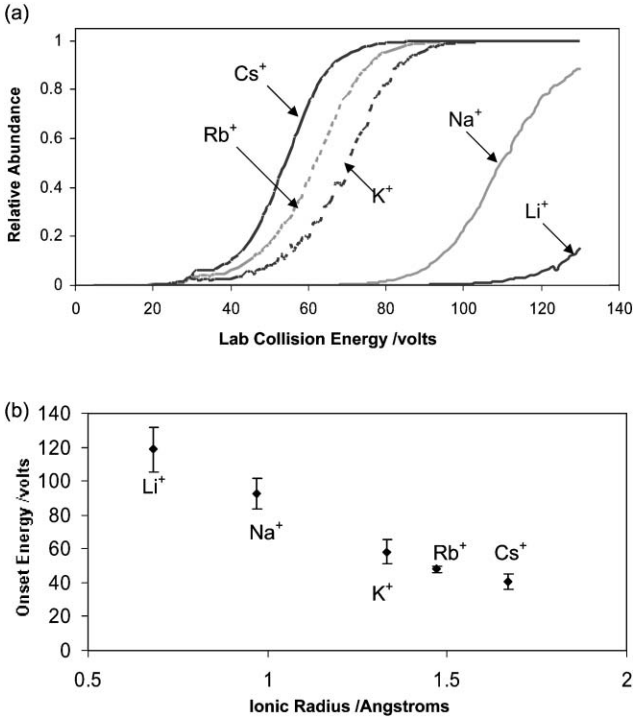


Fig. 2 Precursor ion $[Mon+M]^+$. (a) Relative abundance of M^+. (b) Onset energy of M^+ down Group I. Error is defined as one standard deviation from mean calculated from four repeated experiments. Note: Ionic radii taken from CRC Handbook.[37]

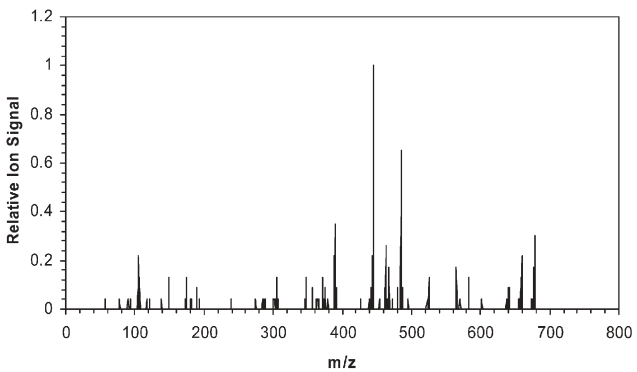
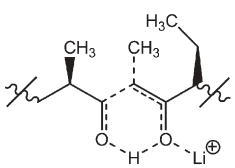


Fig. 3 Example MS/MS spectra of $[Mon+Li]^+$ (mass 677.4) at 90 V collision energy.



Scheme 4 Proposed six-membered ring structure of $[Las+Li]^+$.

A possible explanation involves lengthening and activation of the C(11)–C(12) bond in the precursor species, due to the presence of Li^+. As the alkali metal cation increases in size, the effective ion–dipole interaction decreases, and as a result, the C(11)–C(12) bond in the parent species will decrease in length, toward a single bond. This trend is observed for $[Las\text{-}Na]^+$, as the onset energy of the Na^+–(F Fragment) is greater than for the lithium derivative (the rearrangement occurs at higher lab CID energy), but this cannot be confirmed for the larger alkali metals because the F fragment ions are only observable over a short energy range which is not long enough to measure an accurate onset energy.

4. Dependence on ionic radius

From a purely qualitative observation of Fig. 1a the onset energy for alkali metalled lasalocid appears to increase with increasing ionic radii,[37] yet by extrapolation this is not found to be so. This phenomenon can be explained by examining the onset energy of the free metal ion fragment (M^+). We note that the M^+ onset curve overlaps with the $[Las+M-236amu]^+$ onset curve for K^+ and Rb^+ species. So that above the M^+ onset energy value, the majority of ions formed in the collision cell will form the free metal ion species. Therefore the values attained for the onset of $[Las+K-236amu]^+$ and $[Las+Rb-236amu]^+$ are the lower limit of the actual onset energy, because the majority of ions formed are the free metal cations, not the $[Las+M-236amu]^+$ cations.

Fig. 4b shows the onset of the free metal ion to be related to the ionic radii when the metal cation is in *a* +1 oxidation state. The relationship is not linear, but it can be deduced that the experimental points approach an absolute value in an exponential type function. The importance of the absolute value is unclear. All valid lasalocid results are tabulated in Table 1.

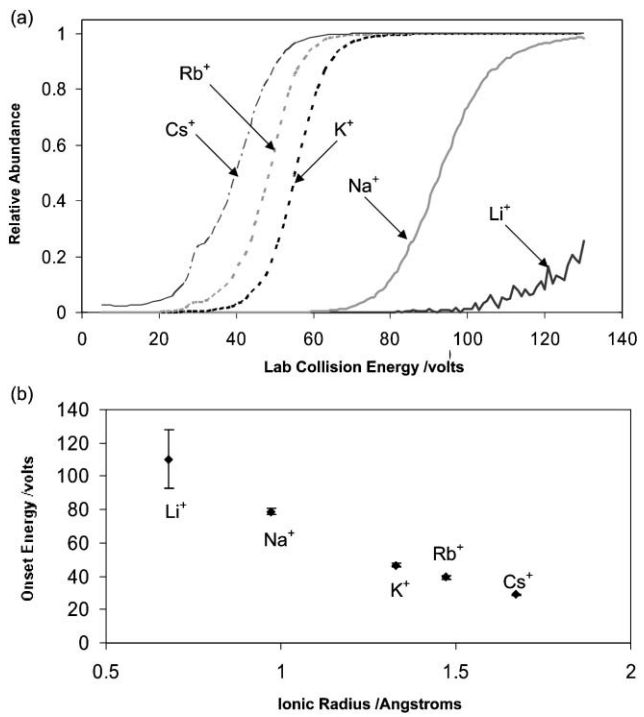


Fig. 4 Precursor ion $[Las+M]^+$. (a) Relative abundance of M^+. (b) Onset energy of M^+ down Group I. Error is defined as one standard deviation from mean calculated from four repeated experiments. Note: Ionic radii taken from CRC Handbook.[37]

Table 2 Experimental data obtained from MRM scans of indicating onset energies of free metal cations

M^+	M^+ ionic radius/Å	Precursor ion $[Las\text{-}M]^+$ onset energy of M^+/V	Precursor ion $[Mon\text{-}M]^+$ onset energy of M^+/V
Li^+	0.68	110.32 (17.65)[a]	118.72 (13.05)
Na^+	0.97	78.80 (1.97)	92.66 (9.27)
K^+	1.33	46.50 (1.34)	58.19 (6.98)
Rb^+	1.47	39.37 (0.89)	48.03 (1.80)
Cs^+	1.67	29.04 (0.39)	40.78 (4.75)

[a] Note: Error values in parentheses (±). Lower limit value. Ionic radii taken from CRC Handbook.[37]

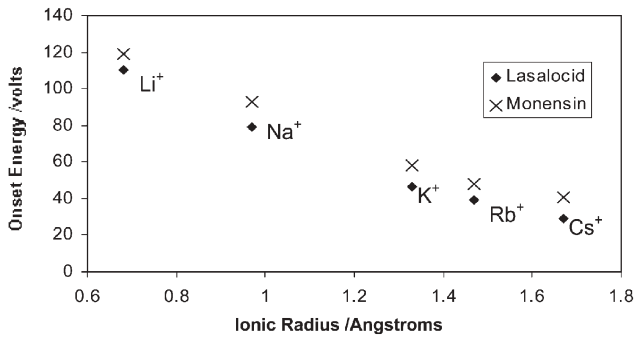


Fig. 5 Comparison of M^+ onset energies for $[Las\text{-}M]^+$ and $[Mon\text{-}M]^+$. Ionic radii taken from CRC Handbook.[37] Error bars are not included for clarity. The actual uncertainties are given in Tables 1 and 2.

The data in Table 2 shows the relative binding abilities of the alkali metal cations to both lasalocid and monensin A. We can infer from this data that in the gas phase monensin A binds stronger to alkali metal cations than lasalocid. This can be observed in Fig. 5. This may be due to the increased rigidity of the monensin A backbone forming a more stable pocket, and the greater amount of hydrogen bonding between monensin A termini over lasalocid. The difference in onset energy is as little as 7.6% for M = Li and as much as 40.4% for M = Cs. Indeed, with the apparent exception of Rb, this difference increases as the ionic radius increases.

The results obtained from these experiments, are not as quantitative as true threshold energy determinations, but can be compared relative to threshold data attained for a similar family of systems. Amunugama and Rodgers[38] determined single-bond threshold energies for alkali pyridyls—$[py\text{-}Li]^+$, $[py\text{-}Na]^+$ and $[py\text{-}K]^+$ (py = pyridine). These values, when plotted against the ionic radii of the free metal ion species, give a similar trend to the data presented here. This type of correlation can also be drawn between other similar work.[39,40]

Concluding remarks

In an attempt to increase the knowledge base of metal affinities and complex stabilities of polyether ionophore antibiotics, we have shown that monensin A has a greater binding affinity for alkali metals than lasalocid. Also the observed F type fragmentation has given insight into remote bond activation by coordination of an alkali metal ion. The results indicate a dependence of the interaction of monensin A and lasalocid with alkali metal cations on their ionic radius and that monensin A binds stronger to alkali metal cations than lasalocid. Further research using different ionophores is currently being undertaken to clarify these observations. The results generally contribute to our ability to identify polyether ionophore antibiotics using electrospray mass spectrometry.

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