Conversion of Ca^{2+} Salt of an Organic Compound to Its Li^+ Salt to Simplify the Fast Atom Bombardment Mass Spectrum

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The FAB mass spectrum of the Ca^{2+} salt of RK-682 (1, MW 368), a potent protein tyrosine phosphatase inhibitor, shows a complex pattern due to Ca^{2+} adduct ions with multimers of 1 and their decomposition ions. Addition of LiCl greatly simplified the FAB mass spectrum, providing a prominent Li^+ adduct ion of 1 at *m/z* 381 $[M+2Li-H]^+$. The addition of LiCl also greatly simplified the FAB mass spectrum of calcium pantothenate. This approach may be generally useful for molecular weight determination of multivalent metal salts of organic compounds, or organic compounds that can form Li salts, by FAB mass spectrometry.

Key words FAB mass spectrum; Li^+ adduct effect; Ca^{2+} salt; multivalent metal salt

Mass spectrometry is a powerful tool to obtain molecular weight (M) and structural information in organic chemistry. However, it is ineffective if a compound does not show significant ion peaks in the mass spectrum. In the field of FAB mass spectrometry,[1,2] several techniques have been developed to generate stable molecular-related ions, including selection of liquid matrices,[3] addition of an alkali metal salt to induce the adduct ion peak $[M+A]^+$ (A: atomic weight of the alkali metal),[4,5] and chemical derivatization to form stable ions.[6] However, even if these techniques are successful, difficulty can arise if a compound forms multiple ions. For example, multivalent metal salts of organic compounds often show complex ion peaks in the spectra, and in such cases, it is difficult to identify the molecular-related ion peaks.[7] We recently experienced such a case during our synthetic studies on RK-682 (**1**), a potent protein tyrosine phosphatase inhibitor, which is converted to the Ca^{2+} salt (**2**) during silica gel column chromatography (Fig. 1).[8,9] The FAB mass spectrum of **2** that we obtained was incomprehensible, since **1** existed as a mixture of Ca^{2+} complexes and their fragment ions. However, we found that the addition of LiCl to **2** was effective to simplify the spectrum, and to confirm the structure. Here, we describe the simplifying effect of Li^+ on the FAB mass spectrum of **2**. To assess whether the effect might be general, we also examined the case of calcium pantothenate.

Results and Discussion

RK-682, a potent protein tyrosine phosphatase inhibitor, was isolated from mycelium of *Streptomyces* sp. 88-682 by a RIKEN group, and the chemical structure was reported to be **1**, though the stereochemistry was not determined at that time (Fig. 1).[10,11] The same structure ((*R*)-**1**) had been assigned by a Takeda group two years before to an inhibitor of phospholipase A_2 which was isolated from cultures of *Streptomyces* sp. AL-462.[12] However, some spectral data of RK-682 were different from those of the latter compound. In order to clarify the structure of RK-682, we synthesized (*R*)- and (*S*)-**1**, and confirmed the stereochemistry of RK-682's asymmetric carbon center at C-5 to be *R*.[8] We also found that RK-682 had been isolated from the natural source as the Ca^{2+} salt, which accounted for the discrepancies between the spectral data of natural RK-682 and those of (*R*)-**1**.[9] RK-682 (**1**) readily binds Ca^{2+}, and synthesized (*R*)-**1** was converted to its Ca^{2+} salt (**2**) during column chromatography on Silica gel 60 (Merck) with MeOH–$CHCl_3$.[9] The salt was formed by reaction of **1** with a small amount of Ca^{2+} contained in Silica gel 60.[13]

FAB mass spectra of RK-682 (**1**) and its Ca^{2+} salt (**2**) are shown in Figs. 2a and b, respectively. The spectrum of **1** shows an intense protonated molecule peak $[M+H]^+$ at *m/z* 369. However, the spectral pattern of **2** is completely different from that of **1**. The presence of calcium in **2** had been proved by X-ray fluorescence analysis, and the amount was determined by ICP-AES (inductively coupled plasma atomic emission spectroscopy).[9] On the basis of high resolution (HR)-FAB-MS data and collisionally activated dissociation (CAD) spectra, the main peaks in the spectrum of **2** were elucidated to be Ca^{2+} adduct ions of the monomer (*m/z* 407, $[M+Ca-H]^+$), dimer (*m/z* 775, $[2M+Ca-H]^+$), trimer (*m/z* 1181, $[3M+2Ca-3H]^+$) and tetramer (*m/z* 1587, $[4M+3Ca-5H]^+$) of **1**, and their fragment ions at *m/z* 377 $[M+Ca-CH_2O-H]^+$ and *m/z* 753 $[2M+2Ca-2CH_2O-3H]^+$.[9]

In general, alkali metal adduct ions $[M+A]^+$ are stable molecular-related ions. Such adduct ions are observed in FAB mass analyses of various organic compounds, including polyoxygenated compounds such as saccharides and polyethers, and polyfunctional compounds such as peptides.[4,5] We previously reported that compounds with two proximal oxygens, and those with a carbonyl group, efficiently coordinate to Na^+.[4,5] We also found that olefinic π-electrons participate in such coordination.[4] Among the alkali metal cations, Li^+ has strong affinity for oxygen atoms.[14] In general, the affinity of alkali metal cations decreases in the order $Li^+ > Na^+ > K^+$,[5,15—17] except for cyclic polyethers, whose binding selectivity depends on the ring size.[18—20] Thus, anticipating the metal exchange of Ca^{2+} in **2** to Li^+, we added a small amount of LiCl to **2**, and measured the FAB mass spectrum of the mixture. The spectrum was greatly simplified, and the Li^+ adduct ion of **1** was observed as a prominent peak at *m/z* 381 $[M+2Li-H]^+$ [Fig. 2c]. It was considered that Li^+ had deaggregated the Ca^{2+}-induced aggregates, and

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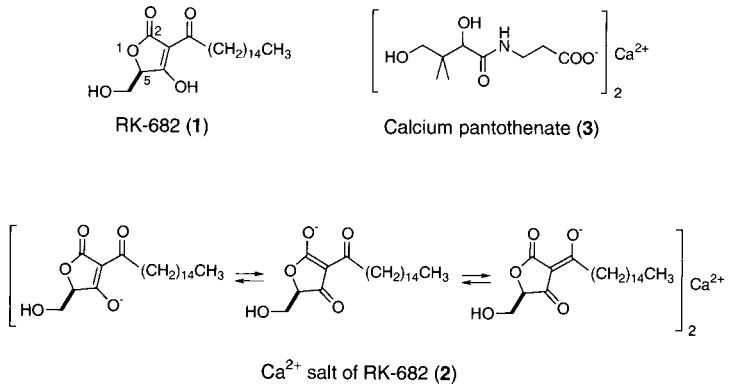

Fig. 1

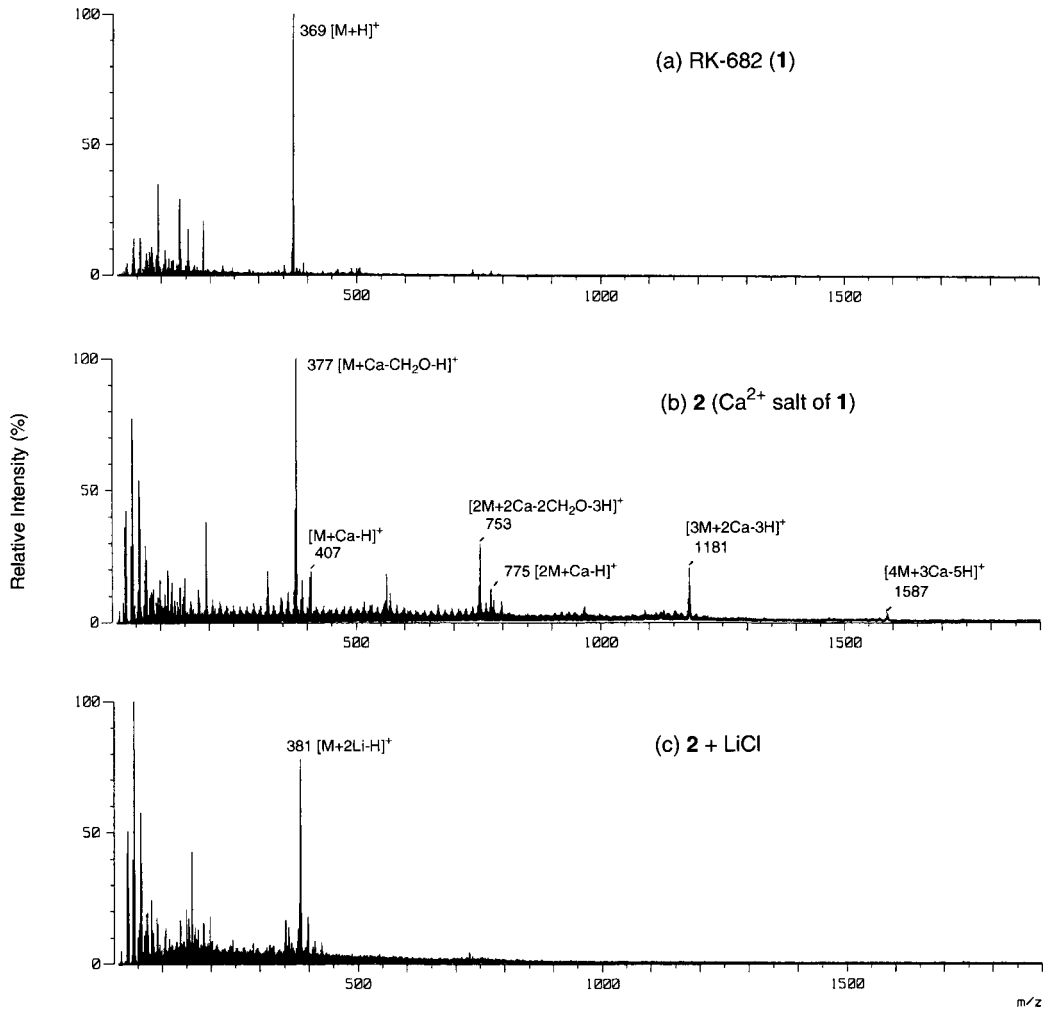

Fig. 2. Positive Ion FAB Mass Spectra

(a) RK-682 (**1**). (b) Compound **2** (Ca^{2+} salt of **1**). (c) Compound **2** with LiCl. Matrices: (a) glycerol–*m*-nitrobenzyl alcohol (2 : 1). (b) DTT–TG11 [dithiothreitol–thioglycerol (1 : 1)]. (c) *m*-nitrobenzyl alcohol.

formed an Li^+ adduct ion in which one acidic hydrogen of **1** had been replaced by Li to form the ion $[M+2Li-H]^+$. From the mass spectrum of the Li^+ adduct ion, the molecular weight of **1** was easily confirmed.

If the complicated FAB mass spectrum of multivalent metal complexes could be simplified in general, it would be very useful for convenient molecular weight determination. Thus, the effect of LiCl was examined with another Ca^{2+} salt, calcium pantothenate (**3**) (Fig. 1), using glycerol as the matrix.[21] The analyses were carried out semi-quantitatively.

Figure 3a shows the FAB mass spectrum of 0.1 M calcium pantothenate (**3**) in glycerol (molecular weight of pantothenic acid (M): 219). The spectrum indicates that calcium pantothenate was dissociated in the glycerol solution, and the

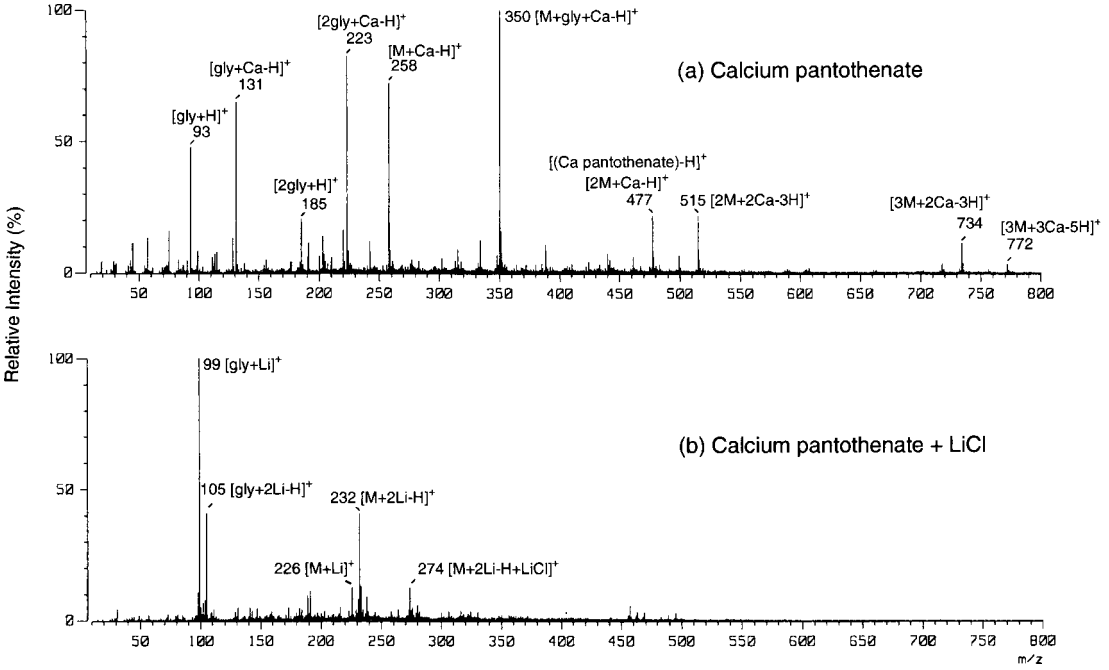

Fig. 3. Positive Ion FAB Mass Spectra

(a) 0.1 M calcium pantothenate. (b) 0.1 M calcium pantothenate with 2 M LiCl. Matrix: glycerol. Molecular weight of pantothenic acid (M: 219), glycerol (gly: 92).

liberated Ca^{2+} formed aggregates with pantothenic acid, glycerol and their multimers. To this solution, a methanolic solution of LiCl was added. As the amount of LiCl was increased, the spectral pattern gradually changed. When the LiCl concentration reached 2 M, all Ca^{2+} adduct ions of pantothenic acid were converted into Li^+ adduct ion, and a prominent ion peak $[M+2Li-H]^+$ was observed at *m/z* 232 [Fig. 3b]. Peaks were also seen at *m/z* 226 and *m/z* 274, corresponding to $[M+Li]^+$ and $[M+2Li-H+LiCl]^+$, respectively.[22] At the concentration of 0.1 M calcium pantothenate in glycerol, the amount of LiCl added to form the ion $[M+2Li-H]^+$ was 10 eq to pantothenic acid. However, because glycerol used as the matrix also binds to Li^+,[23] most of the LiCl added had been consumed to form Li^+ adduct ions of glycerol $[M+Li]^+$ (*m/z* 99) and $[M+2Li-H]^+$ (*m/z* 105). Interestingly, both pantothenic acid and glycerol were converted into the Li^+ adduct ions of their monomers.

These results indicated that, in a similar manner to the Ca^{2+} salt **2**, calcium pantothenate (**3**) was converted into the Li^+ salt with LiCl. NaCl similarly converted the Ca^{2+} salt **3** to the Na^+ salt, though the intensity of the adduct ion was lower (data not shown). LiCl is superior to NaCl from a practical point of view, since the atomic weight of Li (atomic weight: 7) is small, and Li^+ adduct ions are easily identified in the spectrum.

In conclusion, we have shown that Li^+ can greatly simplify the FAB mass spectra of multivalent metal salts. A structurally informative Li^+ adduct ion can be obtained simply by adding a small amount of aqueous or methanolic LiCl solution to a sample on the target tip for FAB mass analyses.

Experimental

Materials Preparation of compounds **1** and **2** was described in a previous paper.[9] Glycerol, *m*-nitrobenzyl alcohol (*m*-NBA), and DTT/TG11 [dithiothreitol, thioglycerol (1 : 1)] were purchased from Tokyo Kasei Kogyo Co., Ltd. Calcium pantothenate and LiCl were purchased from Wako Pure Chemical Industries, Ltd.

Instrumentation and Sample Preparation FAB mass spectra were recorded on a JEOL JMS-HX110 double-focusing mass spectrometer of EBE arrangement with a JMS-DA7000 data system. The ion acceleration voltage was 10 kV, and xenon gas was accelerated at a voltage of 6 kV. Glycerol, *m*-NBA, and DTT/TG11 were used as matrices. LiCl was used as the Li^+ cation source.

FAB mass analyses of compounds **1** and **2** were described in the previous paper.[9] A mixture of **2** and LiCl for FAB mass analysis was prepared by the addition of methanolic LiCl solution to a mixture of **2** in tetrahydrofuran and *m*-NBA on the target tip for FAB mass analyses. Calcium pantothenate solution for FAB mass analysis was prepared by mixing 5 ml each of glycerol and 0.1 M calcium pantothenate in H_2O. Calcium pantothenate solutions containing various amount of LiCl were prepared by mixing 5 μl each of 0.1 M Ca pantothenate in H_2O, 0.1—4 M LiCl in CH_3OH, and glycerol. An aliquot of the mixture was applied to the target tip. FAB mass spectra were obtained by means of a 5.2 s scan from *m/z* 10 to 1900 at 10 s intervals. Three spectra, recorded at 20, 30, and 40 s from the start of the scanning, were averaged. The FAB mass spectrum of each sample was measured at least twice.

References and Notes

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