Coordination of Divalent Metal Cation to Amide Group to Form Adduct Ion in FAB Mass Spectrometry: Implication of Zn^{2+} in Enzymatic Hydrolysis of Amide Bond

Hisayoshi KOBAYASHI, Naoko MORISAKI, Hiroyuki MIYACHI, and Yuichi HASHIMOTO*

Institute of Molecular & Cellular Biosciences, The University of Tokyo; 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–0032, Japan.
Received December 21, 2007; accepted February 13, 2008; published online February 14, 2008

The structures of complexes between amides and metal ions were examined by FAB mass spectrometry and collision-induced dissociation (CID). Zn^{2+} was coordinated by the amide carbonyl oxygen atom of *N*-tetradecylacetamide (1). In contrast, Ca^{2+} and Mg^{2+} were coordinated by the amide group of 1 in both the keto and enol forms. The catalytic role of Zn^{2+} at the active site of the hydrolases might partly be explained by the effective attack of Zn^{2+} on carbonyl oxygen atom of the scissile amide group.

Key words zinc ion (Zn^{2+}); amide group; adduct ion; FAB mass spectrometry; hydrolase

The zinc ion (Zn^{2+}) plays an important role as the catalyst in the enzymatic hydrolysis of amide bonds. For example, Zn^{2+} is essential at the active site of metalloproteinases, such as carboxypeptidases, thermolysins and collagenases.[1—6] It is also essential for the activity of histone deacetylases (HDACs), which catalyze the deacetylation of ε-*N*-acetyl lysine residues of nucleosomal histones and regulate gene expression.[7—10] It is well known that HDAC inhibitors are potential anticancer agents, as they cause growth arrest, differentiation and/or apoptosis in various tumor cell types.[11,12] Almost all HDAC inhibitors contain a functional group that binds to Zn^{2+}, *e.g.*, disulfide, thiol, hydroxamic acid or epoxyketone.[11—13] The phthalimide-type and cyclic amide-type HDAC inhibitors that we have synthesized are hydroxamic acid derivatives.[14,15]

In the crystal structure of the enzyme, the catalytic Zn^{2+} is coordinated by amino acid side chains. X-ray crystallography revealed that, in carboxypeptidases and thermolysins, Zn^{2+} is coordinated by two His, one Glu and one H_2O,[2,4] in collagenases and gelatinases, it is coordinated by three His,[6] and in HDAC8 and in HDAC homologue, by two Asp and one His and one H_2O.[8,10] At the cleavage of an amide bond, the Lewis acidic Zn^{2+} at the active site of the enzyme is believed to promote the cleavage by coordination of the amide carbonyl oxygen atom.[2,5,8,16—18] A proposed mechanism for the first step of the Zn^{2+}-catalyzed hydrolysis of the amide bond of acetylated lysine by HDAC homologue is shown in Fig. 1.[8] Coordination of the carbonyl oxygen atom to Zn^{2+} is followed by nucleophilic attack of the Zn^{2+}-bound water.

A selective requirement of Zn^{2+} for the enzyme activity was demonstrated by means of reconstitution experiments with HDAC homologue.[8] The *in vitro* deacetylase activity was observed after incubation of the purified HDAC homologue with zinc chloride.[8] No significant activity was observed with HDAC homologue preparations reconstituted with Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+} or Mn^{2+}.[8] These results suggest that the behavior of Zn^{2+} in relation to an amide group might be different from that of other metal ions. Therefore, it is of interest to investigate how Zn^{2+} and other metal ions interact with an amide group.

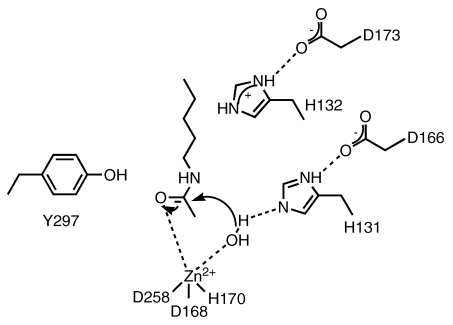

Fig. 1. Proposed Mechanism for the First Step of HDAC Deacetylation[8]

FAB mass spectrometry is a powerful tool to analyze adduct ions formed by coordination of organic compounds to metal ions. When a mixture of an organic compound and a metal salt in matrix solution is bombarded with fast atoms (*e.g.*, Xe), the intact adduct ions and fragment ions are desorbed. Singly charged ions are mainly detected in FAB ionization.[19—21] We have studied the structural requirements (oxygen functional groups and olefinic double bond) for the coordination of metal ions by means of FAB mass spectrometry, and applied the results to the structural elucidation of organic compounds.[22—26] Under FAB conditions, a carbonyl oxygen atom and at least two proximal oxygen atoms efficiently coordinate to a metal cation, and π-electrons participate to form adduct ions. An isolated oxygen atom of a hydroxyl group or an ether hardly forms an adduct ion with a metal cation.[22,23,25]

This paper describes FAB mass analyses of adduct ions of amide group and Zn^{2+} in order to clarify the mode of coordination. The spectral data were compared with those obtained with Ca^{2+}, Mg^{2+} and Co^{2+}. Interactions of peptides with alkaline earth metal ions and transition metal ions, including Zn^{2+}, have been extensively characterized chemically and by mass spectrometry.[27—31] Those findings indicate that a metal ion can bind to several functional groups, such as C-terminal carboxylate, N-terminal amino group, an intervening amide group, and/or amino acid side chain. However, little is known about the interactions of metal ions with a simple amide group.

Results and Discussion

In order to analyze the adduct ions of an amide and Zn^{2+} by FAB mass spectrometry, *N*-tetradecylacetamide (**1**, molecular weight M_1: 255) was prepared (Fig. 2). The positive ion

* To whom correspondence should be addressed. e-mail: hashimot@iam.u-tokyo.ac.jp

FAB mass spectrum of **1** in *m*-nitrobenzyl alcohol (matrix, *m*NBA) showed a strong $(M_1+H)^+$ peak at *m/z* 256 and a weak $(2M_1+H)^+$ peak at *m/z* 511 (data not shown). The adduct ions of **1** and Zn^{2+} were formed by mixing **1**, 0.5 eq of $ZnCl_2$ and *m*NBA. Figure 3A shows the FAB mass spectrum of the mixture. As described above, singly charged ions are formed by FAB ionization, and Zn^{2+} coordination products were detected as $ZnCl^+$ adduct ions.[23] Thus, a strong $(M_1+ZnCl)^+$ peak at *m/z* 354 (a_1), an $(2M_1+ZnCl)^+$ peak at *m/z* 609 (a_2), and a weak $(3M_1+ZnCl)^+$ peak at *m/z* 864 (a_3) were observed in the spectrum. The $ZnCl^+$ adduct ions of **1** are designated as a_n, where the numerical subscript *n* refers to the number of the amide **1** in the adduct ions. A mixture of **1** and $ZnCl_2$ gave only ions of the type $(nM_1+ZnCl)^+$. The isotopic abundance ratio of Zn for masses 64 : 66 : 67 : 68 : 70 is 100 : 57.4 : 8.4 : 38.6 : 1.3, and that of Cl for masses 35 : 37 is 100 : 32.0. Therefore, the spectral pattern of Zn-containing ions, especially that of $ZnCl^+$ adduct ion, is characteristic, and the presence of these ions can be readily recognized in mass spectra.

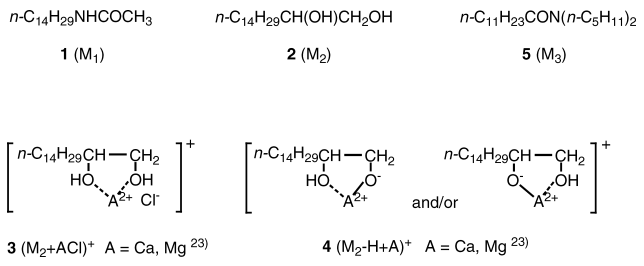

Fig. 2. Structures of the Amides **1**, **5**, the Diol **2** and the Metal Adduct Ions of **2** (**3**, **4**)

Molecular weight and masses of ions are shown in parentheses.

Formation of stable adduct ions between **1** and $ZnCl^+$ under FAB conditions was shown by the time-course of the intensity of the adduct ions during the FAB mass analysis. Rapid decrease of the intensity of the ions a_1 $(M_1+ZnCl)^+$ and a_2 $(2M_1+ZnCl)^+$ was not observed during the analysis from 0 to 5 min. The stability of the ion a_1 was further confirmed by the collision-induced dissociation (CID) spectra of the ions a_2 (*m/z* 609, $2M_1+{}^{64}Zn^{35}Cl$) and a_1 (*m/z* 354, $M_1+{}^{64}Zn^{35}Cl$) (Figs. 4A, B). The CID spectra show degraded ions produced by the bombardment of the precursor ion with He gas.[32,33] Figure 4A shows that the ion a_2 afforded the ion a_1, and Fig. 4B shows that the ion a_1 did not generate any degraded ion or fragment ion. These data suggest that Zn^{2+} was bound only to the carbonyl oxygen atom of the keto form of **1**. As will be described below, deprotonated ions may be

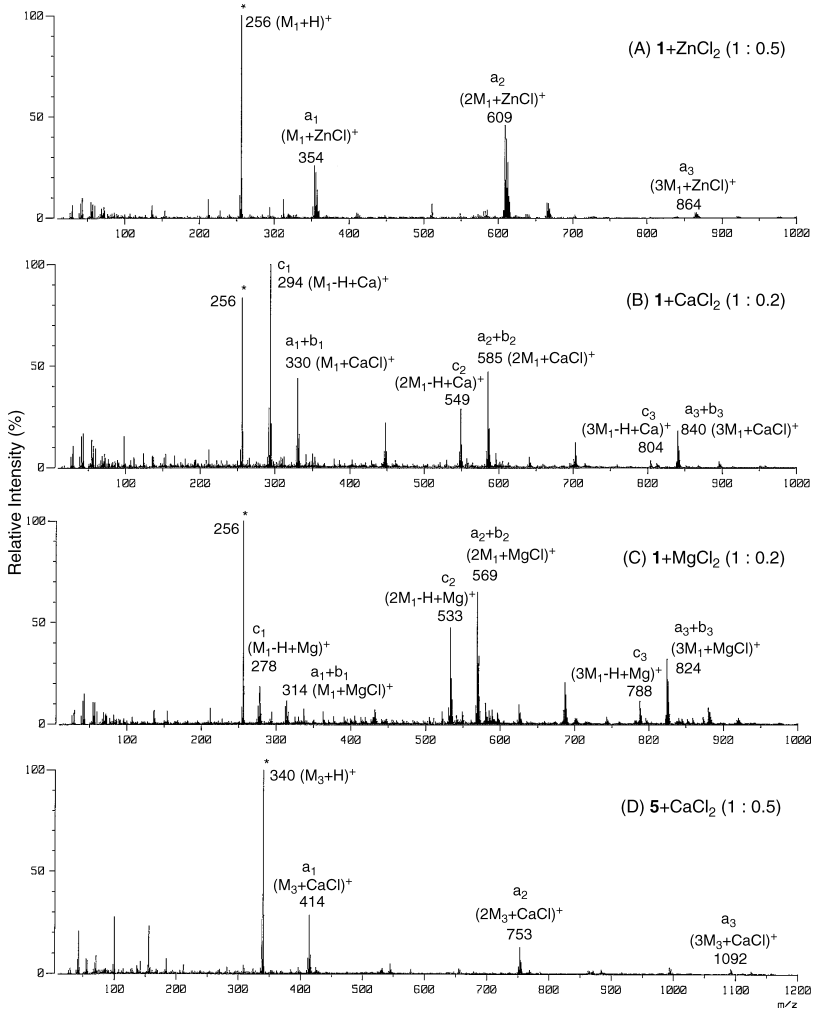

Fig. 3. Positive ion FAB-MS

(A) **1** with 0.5 eq of $ZnCl_2$, (B) **1** with 0.2 eq of $CaCl_2$, (C) **1** with 0.2 eq of $MgCl_2$, (D) **5** with 0.5 eq of $CaCl_2$. Protonated molecule $(M+H)^+$. Matrix: *m*-nitrobenzyl alcohol. Ion peaks are designated as follows. a_n: keto form amide complex, b_n: enol form amide complex, c_n: deprotonated amide complex.

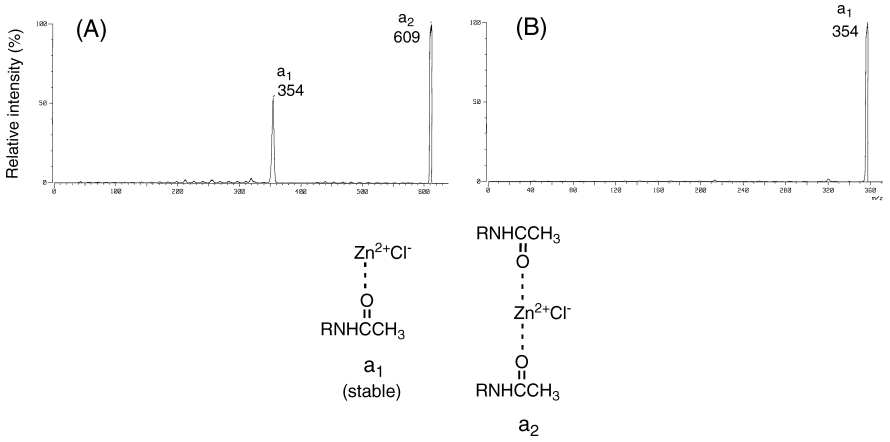

Fig. 4. CID Spectra of (A) a_2: $(2M_1+{}^{64}Zn^{35}Cl)^+$ and (B) a_1: $(M_1+{}^{64}Zn^{35}Cl)^+$, and Plausible Structures of a_1 and a_2

formed from the adduct ions containing the enol form of **1**. Plausible structures of a_1 and a_2 are shown in Fig. 4.

In contrast to Zn^{2+}, we found that Ca^{2+} and Mg^{2+} displayed different behavior with the amide group. Figure 3B shows the FAB mass spectrum of a mixture of **1** and 0.2 eq of $CaCl_2$. The spectrum shows $CaCl^+$ adduct ions a_1: $(M_1+CaCl)^+$ at *m/z* 330 (Calcd for $C_{16}H_{33}NOCa^{35}Cl$: 330.1877, Found: 330.1837), a_2: $(2M_1+CaCl)^+$ at *m/z* 585 and a_3: $(3M_1+CaCl)^+$ at *m/z* 840. Ions b_1, b_2 and b_3, the $CaCl^+$ adduct ions of the enol form of **1**, will be described below. A remarkable feature of the spectral pattern is the relatively high abundance of the ions c_1, c_2 and c_3. Deprotonated and Ca^{2+} adduct ions c_1: $(M_1-H+Ca)^+$, c_2: $(2M_1-H+Ca)^+$ and c_3: $(3M_1-H+Ca)^+$ were formed at *m/z* 294 (Calcd for $C_{16}H_{32}NOCa$: 294.2110, Found: 294.2073), 549 and 804, respectively. The masses of these ions c_n formally correspond to the $CaCl^+$ adduct ion of **1** minus HCl. The FAB mass spectrum of the mixture of **1** and 0.2 eq of $MgCl_2$ also gave $MgCl^+$ adduct ions, and deprotonated and Mg^{2+} adduct ions (Fig. 3C). Above-mentioned isotope distribution of zinc causes relatively low abundance of Zn-containing ions. Therefore, $ZnCl^+$ adduct ions of **1** in Fig. 3A were formed with 0.5 eq of $ZnCl_2$. The relative intensities of the adduct ions a_1, a_2, a_3 in Fig. 3A and those in the spectrum of **1** with 0.2 eq of $ZnCl_2$ were essentially the same (data not shown).

Our previous study showed that the FAB mass spectrum of a mixture of 1,2-hexadecanediol (**2**, molecular weight: M_2) and $CaCl_2$ gave $CaCl^+$ adduct ions $(M_2+CaCl)^+$ (**3**) and $(2M_2+CaCl)^+$, and deprotonated and Ca^{2+} adduct ion $(M_2-H+Ca)^+$ (**4**) (Fig. 2).[23] Deprotonation had occurred at a hydroxyl group of **2**, and $MgCl_2$ displayed similar behavior to $CaCl_2$.[23] The FAB mass spectrum of a mixture of the diol **2** and $ZnCl_2$ also showed the ion $(M_2-H+Zn)^+$ (data not shown). Hall and Brodbelt studied the adduct ions of diketones and metal ions by electrospray ionization (ESI) mass spectrometry, and reported on the influence of keto-enol tautomerism of diketones and their chelation with alkaline earth metal ions and transition metal ions.[34] They found that deprotonated and metal adduct ions of the type $(2L-H^++M^{2+})^+$ (L: enolizable 1,3-diketone, M: metal) were formed through displacement of a proton in the enol form of the diketone upon coordination of the metal ion. From our results on the metal adduct ions of the diol **2** and the reported data on 1,3-diketones,[34] it seems reasonable to consider that Ca^{2+} and Mg^{2+} were coordinated with the amide group of **1** in both the keto and enol forms, and that deprotonation occurred at the hydroxyl group of the enol form of the amide group. Plausible structures of these Ca^{2+} adduct ions are shown in Fig. 5. $CaCl^+$ adduct ions of keto and enol forms of the amide **1** are assigned to be a_n and b_n, respectively. Only one structure is shown for each ion, although several forms are possible.

In order to confirm these structures, CID experiments were carried out with the Ca^{2+} adduct ions of **1** (Fig. 5). The ion (a_1+b_1): $(M_1+Ca^{35}Cl)^+$ at *m/z* 330 gave a fragment ion c_1: $(M_1-H+Ca)^+$ at *m/z* 294, which showed that the precursor ion contained the enol form of the amide group (b_1), and the ion b_1 gave the ion c_1 by elimination of HCl (Fig. 5A). The ion c_1 at *m/z* 294 stayed unchanged, which showed that c_1 was stable (Fig. 5B). The ion (a_2+b_2): $(2M_1+Ca^{35}Cl)^+$ at *m/z* 585 gave the ions a_1 and c_1, which showed that the precursor ion contained keto and enol forms of the amide **1** (Fig. 5C). Presumably, a_1 and c_1 were formed from a_2 and b_2, respectively. The ion c_2: $(2M_1-H+Ca)^+$ at *m/z* 549 gave only the stable ion c_1 by elimination of a molecule of **1** (Fig. 5D). These data showed that Ca^+ formed adduct ions with **1** in both keto and enol forms.

In order to examine the FAB mass spectrum of non-enolizable amide complex, *N*,*N*-dipentyldodecanamide (**5**) (molecular weight: M_3) was prepared, and the FAB mass spectrum of a mixture of **5** and 0.5 eq of $CaCl_2$ was recorded (Fig. 3D). As expected, the spectrum showed only $CaCl^+$ adduct ions of the keto form of **5**, a_1: $(M_3+CaCl)^+$ at *m/z* 414, a_2: $(2M_3+CaCl)^+$ at *m/z* 753 and a_3: $(3M_3+CaCl)^+$ at *m/z* 1092, and the spectral pattern was similar to that of a mixture of **1** and $ZnCl_2$ (Fig. 3A). These data further support our conclusion that Zn^{2+} is coordinated by amide group only in the keto form.

It was reported that, in the reconstitution experiments of purified HDAC homologue, Co^{2+} produced a comparable level of activity with Zn^{2+}.[8] Co^{2+} can often substitute for Zn^{2+} in enzymatic experiments.[1,8] Therefore, we tried to analyze a mixture of the amide **1** and $CoCl_2$ by FAB mass spectrometry. However, Co^{2+} adduct ions of **1** were unstable under FAB conditions, and the ions formed rapidly disappeared during the analysis. Only the first scan of a mixture of **1** and $CoCl_2$ (1 : 5) gave a spectrum of Co^{2+} adduct ions (Fig.

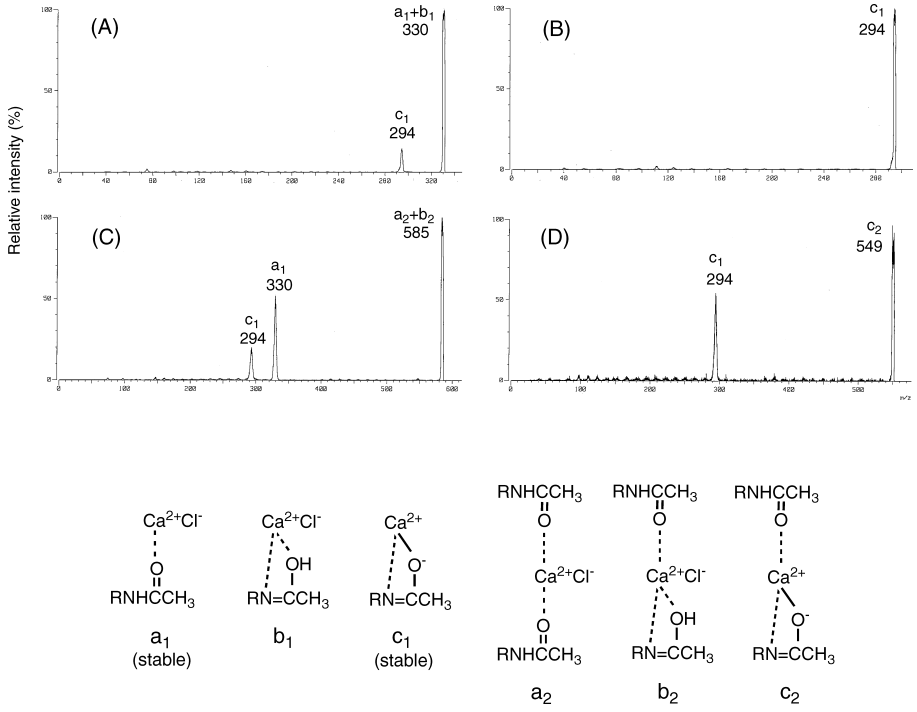

Fig. 5. CID Spectra of (A) (a_1+b_1): $(M_1+Ca^{35}Cl)^+$, (B) c_1: $(M_1-H+Ca)^+$, (C) (a_2+b_2): $(2M_1+Ca^{35}Cl)^+$ and (D) c_2: $(2M_1-H+Ca)^+$, and Plausible Structures of the Ions a_1, a_2, b_1, b_2, c_1 and c_2

Only one structure is shown for each ion, although several forms are possible.

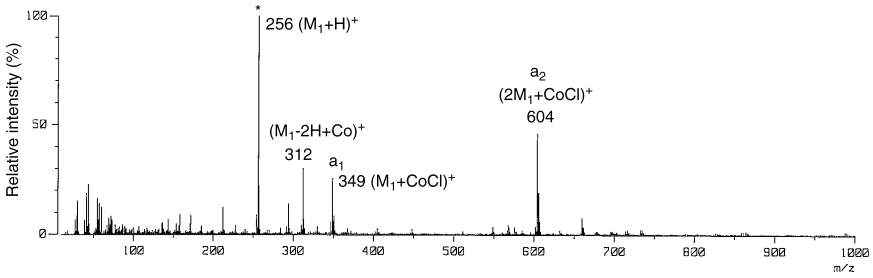

Fig. 6. Positive Ion FAB-MS of **1** with 5 eq of $CoCl_2$

Matrix, *m*-nitrobenzyl alcohol.

6). It showed $CoCl^+$ adduct ions a_1: $(M_1+CoCl)^+$ at *m/z* 349 and a_2: $(2M_1+CoCl)^+$ at *m/z* 604, and the spectral pattern was similar to that of **1** and $ZnCl_2$. These results indicate that Co^{2+} was bound to the keto form of **1** in a similar manner to Zn^{2+}. A unique ion at *m/z* 312 contained Co^{3+} ion, and was assigned to $(M_1-2H^++Co^{3+})^+$ (Calcd for $C_{16}H_{31}NOCo$: 312.1738, Found: 312.1756). Rapid decrease of $CoCl^+$ adduct ions might be caused by the conversion of Co^{2+} to Co^{3+} under FAB conditions.

In summary, FAB mass analyses showed that the amide **1** formed adduct ions with Zn^{2+} and Co^{2+} in the keto form, and with Ca^{2+} and Mg^{2+} in both the keto and enol forms. The specific role of Zn^{2+} in amide bond hydrolases might be at least partly explained by the effective attack of Zn^{2+} on the carbonyl oxygen atom of the scissile amide group. The high vacuum solvent-free environment of the mass spectrometer provides ideal medium for the investigation of the intrinsic interactions between a metal ion and an amide. The gas-phase interactions may mimic hydrophobic metal ion binding that occurs in interiors of proteins.[35,36]

Experimental

Instrumentation and Sample Preparation FAB mass spectra were recorded on a JEOL JMS-HX110 double-focusing mass spectrometer in an 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. *m*-Nitrobenzyl alcohol (*m*NBA) was used as the matrix. CID spectra were obtained with helium as the collision gas, which was metered to cause about 20% attenuation of the main beam.

Sample solutions for the FAB mass analyses, *e.g.* compound **1** containing 0.5 eq of $ZnCl_2$, were prepared by mixing 5 ml each of 0.1 M compound **1** in $CHCl_3$–CH_3OH (1 : 1), 0.05 M $ZnCl_2$ in H_2O–CH_3OH (1 : 9) and *m*NBA.[22,23,25] An aliquot of the mixture was applied to the target tip for FAB mass analyses. FAB mass spectra were obtained by means of a 5.2 s scan from *m/z* 10 to 1900. The values at 20, 30, and 40 s from the start of the scanning were averaged except for the spectrum of a mixture of **1** and $CoCl_2$ (Fig. 6), which shows the value of the first scan.

Materials Compounds prepared were characterized by ^{1}H-NMR spectroscopy (JEOL JNM A-500 NMR spectrometer, 500 MHz) and FAB mass spectrometry. 1-Tetradecylamine, dipentylamine, $CaCl_2$, $MgCl_2\cdot 6H_2O$, $CoCl_2\cdot 6H_2O$ were purchased from Wako Pure Chemical Industries, Ltd. *n*-Dodecanoyl chloride and *m*NBA, and $ZnCl_2$ were purchased from Tokyo Kasei Kogyo Co., Ltd. and Kanto Chemical Co., Inc., respectively.

***N*-Tetradecylacetamide (1)** To a solution of acetic anhydride (0.5 ml) and pyridine (2 ml) was added a solution of 1-tetradecylamine (220 mg,

1.0 mmol) in benzene (0.5 ml), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2–ethyl acetate 1 : 1) to give **1** (164 mg, 64%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22—1.32 (22H, m), 1.48 (2H, m), 1.97 (3H, s), 3.23 (2H, q), 5.41 (1H, s), FAB-MS: m/z 256 $(M+H)^+$, 511 $(2M+H)^+$.

***N*,*N*-Dipentyldodecanamide (5)** To a solution of dipentylamine (236 mg, 1.5 mmol), pyridine (2 ml) and triethylamine (0.5 ml) was added *n*-dodecanoyl chloride (236 mg, 1.1 mmol) at 0 °C, and the mixture was stirred overnight at room temperature. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane–ethyl acetate 4 : 1) to give **5** (235 mg, 68%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.85—0.97 (9H, m), 1.10 (1H, m), 1.22—1.35 (24H, m), 1.46—1.65 (5H, m), 2.27 (2H, q), 3.15—3.30 (4H, m), FAB-MS: m/z 340 $(M+H)^+$.

Acknowledgement We are grateful to Dr. Aya Tanatani of Department of Chemistry, Ochanomizu University for useful discussions.

References

1) Holmes M. A., Matthews B. W., *Biochemistry*, **20**, 6912—6920 (1981).
2) Matthews B. W., *Acc. Chem. Res.*, **21**, 333—340 (1988).
3) Christianson D. W., Lipscomb W. N., *Acc. Chem. Res.*, **22**, 62—69 (1989).
4) Vallee B. L., Auld D. S., *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 220—224 (1990).
5) Lovejoy B., Hassell A. M., Luther M. A., Weigl D., Jordan S. R., *Biochemistry*, **33**, 8207—8217 (1994).
6) Babine R. E., Bender S. L., *Chem. Rev.*, **97**, 1359—1472 (1997).
7) Grunstein M., *Nature* (London), **389**, 349—352 (1997).
8) Finnin M. S., Donigian J. R., Cohen A., Richon V. M., Rifkind R. A., Marks P. A., Breslow R., Pavletich N. P., *Nature* (London), **401**, 188—193 (1999).
9) Grozinger C. M., Schreiber S. L., *Chem. Biol.*, **9**, 3—16 (2002).
10) Vannini A., Volpari C., Filocamo G., Casavola E. C., Brunetti M., Renzoni D., Chakravarty P., Paolini C., Francesco R. D., Gallinari P., Steinkühler C., Marco S. D., *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 15064—15069 (2004).
11) Nakajima H., Kim Y. B., Terano H., Yoshida M., Horinouchi S., *Exp. Cell Res.*, **241**, 126—133 (1998).
12) Jung M., *Curr. Med. Chem.*, **8**, 1505—1511 (2001).
13) Remiszewski S. W., Sambucetti L. C., Atadja P., Bair K. W., Cornell W. D., Green M. A., Howell K. L., Jung M., Kwon P., Trogani N., Walker H., *J. Med. Chem.*, **45**, 753—757 (2002).
14) Shinji C., Nakamura T., Maeda S., Yoshida M., Hashimoto Y., Miyachi H., *Bioorg. Med. Chem. Lett.*, **15**, 4427—4431 (2005).
15) Shinji C., Maeda S., Imai K., Yoshida M., Hashimoto Y., Miyachi H., *Bioorg. Med. Chem.*, **14**, 7625—7651 (2006).
16) Schepartz A., Breslow R., *J. Am. Chem. Soc.*, **109**, 1814—1826 (1987).
17) Neverov A. A., Montoya-Pelaez P. J., Brown R. S., *J. Am. Chem. Soc.*, **123**, 210—217 (2001).
18) Rivas J. C. M., Salvagni E., Parsons S., *Dalton Trans.*, **2004**, 4185—4192 (2004).
19) Adams J., Gross M. L., *J. Am. Chem. Soc.*, **108**, 6915—6921 (1986).
20) Grese R. P., Cerny R. L., Gross M. L., *J. Am. Chem. Soc.*, **111**, 2835—2842 (1989).
21) Hofmeister G. E., Zhou Z., Leary J. A., *J. Am. Chem. Soc.*, **113**, 5964—5970 (1991).
22) Morisaki N., Inoue K., Kobayashi H., Shirai R., Morisaki M., Iwasaki S., *Tetrahedron*, **52**, 9017—9024 (1996).
23) Morisaki N., Inoue K., Iwasaki S., *J. Mass Spectrom. Soc. Jpn.*, **45**, 471—477 (1997).
24) Sodeoka M., Sampe R., Kojima S., Baba Y., Morisaki N., Hashimoto Y., *Chem. Pharm. Bull.*, **49**, 206—212 (2001).
25) Morisaki N., Kobayashi H., Yamamura Y., Morisaki M., Nagasawa K., Hashimoto Y., *Chem. Pharm. Bull.*, **50**, 935—940 (2002).
26) Morisaki N., Kobayashi H., Nagasawa K., Baba Y., Sodeoka M., Hashimoto Y., *Chem. Pharm. Bull.*, **51**, 1341—1344 (2003).
27) Zhao H., Reiter A., Teesch L. M., Adams J., *J. Am. Chem. Soc.*, **115**, 2854—2863 (1993).
28) Hu P., Gross M. L., *J. Am. Chem. Soc.*, **114**, 9153—9160 (1992).
29) Hu P., Loo J. A., *J. Am. Chem. Soc.*, **117**, 11314—11319 (1995).
30) Cerda B. A., Cornett L., Wesdemiotis C., *Int. J. Mass Spectr.*, **193**, 205—226 (1999).
31) Sigel H., Martin R. B., *Chem. Rev.*, **82**, 385—426 (1982).
32) Biemann K., Scoble H. A., *Science*, **237**, 992—998 (1987).
33) Gillece-Castro B. L., Burllingame A. L., "Methods in Enzymology," Vol. 193, ed. by McCloskey J. A., Academic Press, Inc., San Diego, 1990.
34) Hall B. J., Brodbelt J. S., *J. Am. Soc. Mass Spectrom.*, **10**, 402—413 (1999).
35) Teesch L. M., Adams J., *J. Am. Chem. Soc.*, **112**, 4110—4120 (1990).
36) Cerda B. A., Hoyau S., Ohanessian G., Wesdemiotis C., *J. Am. Chem. Soc.*, **120**, 2437—2448 (1998).