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Kojic acid–amino acid amide metal complexes and their melanogenesis inhibitory activities

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Tyrosinase plays a critical role in the early stages of the melanin synthetic pathway by catalyzing the oxidation of the substrate. Therefore, tyrosinase inhibitors have been intensively studied in both cosmetic and food industries to develop hypopigmentary agents and prevent enzymatic browning in food. Previously, we reported that kojic acid–amino acid amide (KA-AA-NH_2) showed enhanced tyrosinase inhibitory activity compared with kojic acid alone, but this was not observed in a cell test because of poor cell permeability. To enhance cell permeability, we prepared copper and zinc complexes of KA-AA-NH_2 and characterized them using FT-IR spectroscopy, ESI-MS spectrometry, and inductively coupled plasma analysis. We then showed that KA-AA-NH_2 copper complexes exhibited melanogenesis inhibitory activity in Mel-Ab cells. Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this paper.

Keywords: kojic acid–amino acid derivatives; metal complex; depigmenting agent; cell permeability

Introduction

Traditionally, preventing skin pigmentation caused by melanogenesis has been a major concern among Asian women because white skin is a symbol of beauty and prosperity in Asia. In Western cultures, skin whitening materials are applied on patches of irregular hyperpigmentation for treatment purposes. Melanin is a dark pigment produced by skin cells called melanocytes in the stratum basale of the epidermis. There are two kinds of melanin, brown-black eumelanin and red-brown pheomelanin. The darker eumelanin is an important target in addressing skin pigmentation issues as expected. Although melanin is synthesized as a natural photoprotectant against ultraviolet rays and is a scavenger of toxic drugs and chemicals, the abnormal accumulation of melanin through stress or the unbalanced release of hormones leads to aesthetic concerns such as freckles, chloasma, ephelide and senile lentigines. Furthermore, undesirable enzymatic browning of food as observed in vegetable, mushroom and fruits have a negative effect on their commercial value.

In the melanin synthetic pathway, tyrosinase is an essential enzyme that catalyzes *o*-hydroxylation of L-tyrosine (monophenolase or cresolase activity) and oxidation of L-3,4-dihydroxyphenylalanine (diphenolase or catecholase activity) in the early stage of melanogenesis. Melanin, which is a polymer comprising numerous smaller component molecules such as 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid, is consequently produced by sequential oxidation and oxidative cyclization.

Tyrosinase is an important study target for the cosmetic, agricultural and pharmaceutical industries. Therefore, extensive development of tyrosinase inhibitors has occurred.

Kojic acid (KA) has been studied as material for skin whitening and as a food additive to prevent enzymatic browning because it acts as a potent metal chelator and scavenger of free radicals [1,2]. However, KA does not exhibit sufficient tyrosinase inhibitory activity at low concentration or offer long-term stability. Therefore, many KA derivatives have been developed to improve properties via modification of the C-7 hydroxyl group into esters [3], hydroxyphenyl ethers [4], thioethers [5], acrylic acid esters [6], glycosides [7] and amide [8] derivatives.

KA peptide and amino acid derivatives have been prepared, showing highly enhanced tyrosinase inhibitory activity after peptide or amino acid conjugation [9,10]. Although these compounds showed excellent tyrosinase inhibitory activity, their lack of cell permeability still posed problems. Cellular internalization of hydrophilic molecules is a formidable challenge because of the cell membrane, which is a necessary biological barrier to block invasion of xenobiotics into the cell. Therefore, therapeutic agents often struggle to be delivered to targeted sites inside the cell because of membrane impermeability. To overcome this, many delivery methods have been studied with strategies based on cationic lipids [11,12], peptide bond modification [13,14] or conjugating with cell-penetrating peptides [15–21].

As an alternative, we prepared metal complexes of KA–phenylalanine amide (KA-F-NH_2), which had the highest tyrosinase inhibitory activity among the 20 KA–amino acid amides (KA-AA-NH_2). We

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demonstrated that the metal complex worked well in the cell by increasing lipophilicity of the molecule by blocking the functional groups that generate hydrogen bonds [22].

In this study, we optimized the conditions for preparing metal complexes of KA-F-NH_2 and characterized their structures by FT-IR spectra, ESI-MS spectrometry and inductively coupled plasma (ICP) analysis. Also, we synthesized various copper or zinc complexes of KA-AA-NH_2 such as KA-W-NH_2, KA-Y-NH_2 and KA-H-NH_2, which showed the highest tyrosinase inhibitory activity next to KA-F-NH_2. Then, we evaluated their melanogenesis inhibitory activity in Mel-Ab cells.

Materials and Methods

Chemicals

Aminomethyl surface-layered polystyrene (AM SURE™) (100–200 mesh, 0.76 mmol/g) resin, 4-hydroxymethylfuran-2(5H)-one 20 ml filtered reactors (Libra tube RT-20 M), Fmoc-L-amino acids, Fmoc-Rink amide linker, BOP and HOBt were purchased from BeadTech Inc. (Seoul, Korea). KA was purchased from TCI Organic Chemicals (Tokyo, Japan). Copper (II) chloride ($CuCl_2$) was purchased from KANTO chemicals (Tokyo, Japan). Zinc (II) acetate ($Zn(OAc)_2$), 1,1′-carbonyldiimidazole (CDI), diisopropylethylamine (DIPEA), thioanisole, ethanedithiol (EDT) and ninhydrin were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N*-Methyl-2-pyrrolidone (NMP), piperidine, methanol, DCM, DMSO, THF and diethyl ether were from Dae-Jung Chemicals (Siheung city, Korea). DMF was from Mallinckrodt Backer (Paris, KY, USA), TFA was purchased from Acros Organics (Morris Plains, NJ, USA) and phenol was from DC Chemical (Seoul, Korea).

Apparatus

^{1}H NMR spectra were recorded by JEOL JNM-LA300 spectrometer (JEOL Ltd., Tokyo, Japan) (300 MHz). KA-AA-NH_2 and KA-FWY-NH_2 were analyzed by HPLC [Young Lin Autochro 2000 (Young Lin Instrument Co. Ltd., Kyounggi-do, Korea) using Waters μBondapak C18 RP column (Waters Corporation, Milford, MA, USA) (125 Å, 10 μm, 3.9 × 150 mm)]. Cu-[KA-AA-NH_2] and Zn-[KA-AA-NH_2] were characterized by Quattro triple quadrupole tandem mass spectrometer (Waters/Micromass), FT-IR spectroscopy instrument (model FT/IR-200, JASCO, Easton, MD, USA) and ICP-atomic emission spectrometer (ICPS-1000IV, Shimadzu Corporation, Kyoto, Japan).

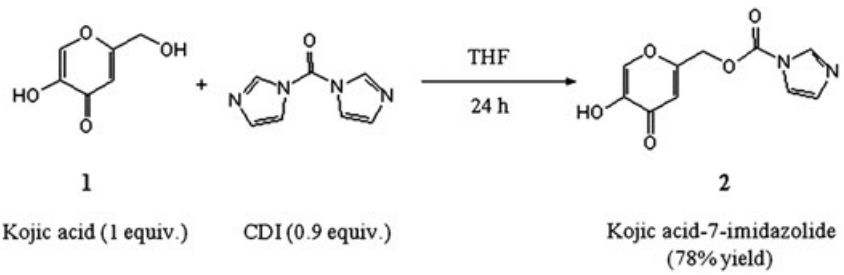

Figure 1. Synthesis of kojic acid-7-imidazolide, compound **2**.

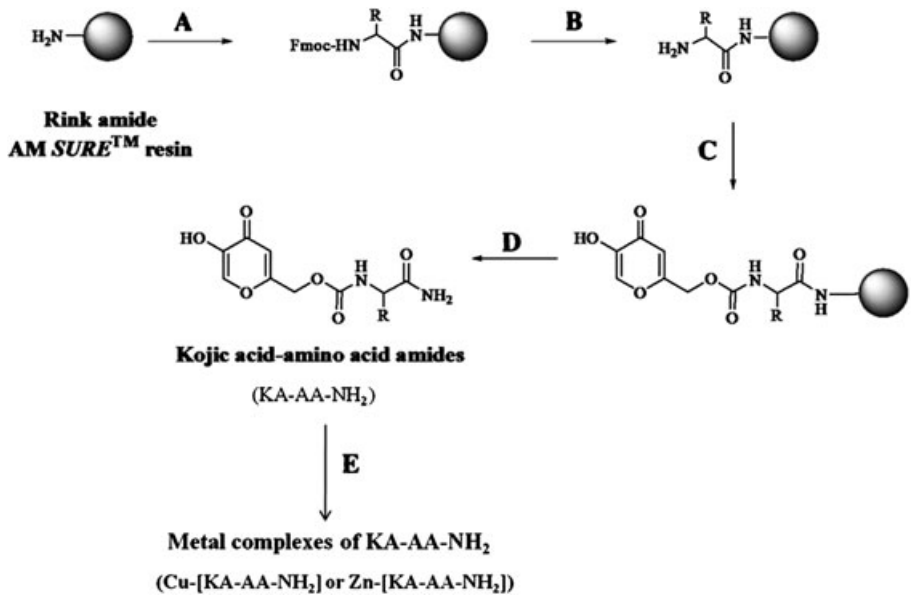

Figure 2. Synthesis of kojic acid–amino acid amides and their metal complexes; Reagents and conditions; (A) Fmoc-L-amino acids (2 equiv.), BOP (2 equiv.), HOBt (2 equiv.) and DIPEA (4 equiv.) in NMP for 1.5 h; (B) 20% piperidine/NMP (v/v) for 3 min and then 15 min; (C) KA-imidazolide (2 equiv.) and HOBt (2 equiv.) in NMP for 6 h; (D) reagent K [TFA/thioanisole/phenol/water/EDT (82.5/5/5/5/2.5, v/v)] for 1 h and diethyl ether precipitation; (E), copper chloride (1 equiv.) or zinc acetate (0.5 equiv.) in water–methanol mixture for 3 h.

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Synthesis of KA-AA-NH_2 Metal Complexes

Kojic acid (5 g, 35 mmol) was dissolved in 100 ml of THF, and then CDI (5.1 g, 31 mmol) was dissolved in 50 ml of THF and added dropwise into magnetically stirred KA solution. After 24 h, a white precipitant, KA-imidazolide (compound **2**) was obtained by filtration.

Selected KA-AA-NH_2, including KA-F-NH_2, KA-W-NH_2, KA-Y-NH_2 and KA-H-NH_2, which showed the highest tyrosinase inhibitory activity among the 20 kinds of KA-AA-NH_2, were manually prepared using the SPPS method with Fmoc chemistry, employing Fmoc-Rink amide AM SURE™ [23]. After removing the Fmoc groups from the Rink amide linker with 20% piperidine/NMP (v/v), *N*-Fmoc-amino acid (2 equiv.) was introduced to the resin using the BOP-mediated coupling method. After removing Fmoc groups from the amino acids, compound **2** (2 equiv.) in NMP was coupled with HOBt as a coupling additive for 6 h. Each coupling and deprotecting step was monitored by the ninhydrin color test. Finally, the resin was treated with reagent K [TFA/thioanisole/phenol/water/EDT (82.5/5/5/5/2.5, v/v)] for 1 h at room temperature to obtain KA-AA-NH_2. The crude KA-AA-NH_2 in the filtrate was concentrated under low pressure and precipitated with cold diethyl ether. The white powder was further washed with diethyl ether (×5) and dried *in vacuo*.

The KA-AA-NH_2 metal complexes were directly prepared from KA-AA-NH_2 and $CuCl_2$ or $Zn(OAc)_2$. KA-AA-NH_2 (0.1 mmol) was dissolved in 10 ml of water or a water–methanol mixture with stirring and gentle heating. To optimize the ratio of KA-AA-NH_2 to metal ion, KA-F-NH_2 was added to aqueous copper solution in different ratios. $CuCl_2$ (0.1 mmol) or $Zn(OAc)_2$ (0.05 mmol) dissolved in 1 ml of water was dropped into the magnetically stirred KA-AA-NH_2 solution, and its pH was adjusted to 6.0 ~ 7.0 with 0.25 *N* NaOH. Copper (II) or zinc (II) complexes of KA-AA-NH_2 were completely formed within 3 h, and those were sedimentary by centrifugation and intensively washed with methanol (×2) and water (×2) to remove salts.

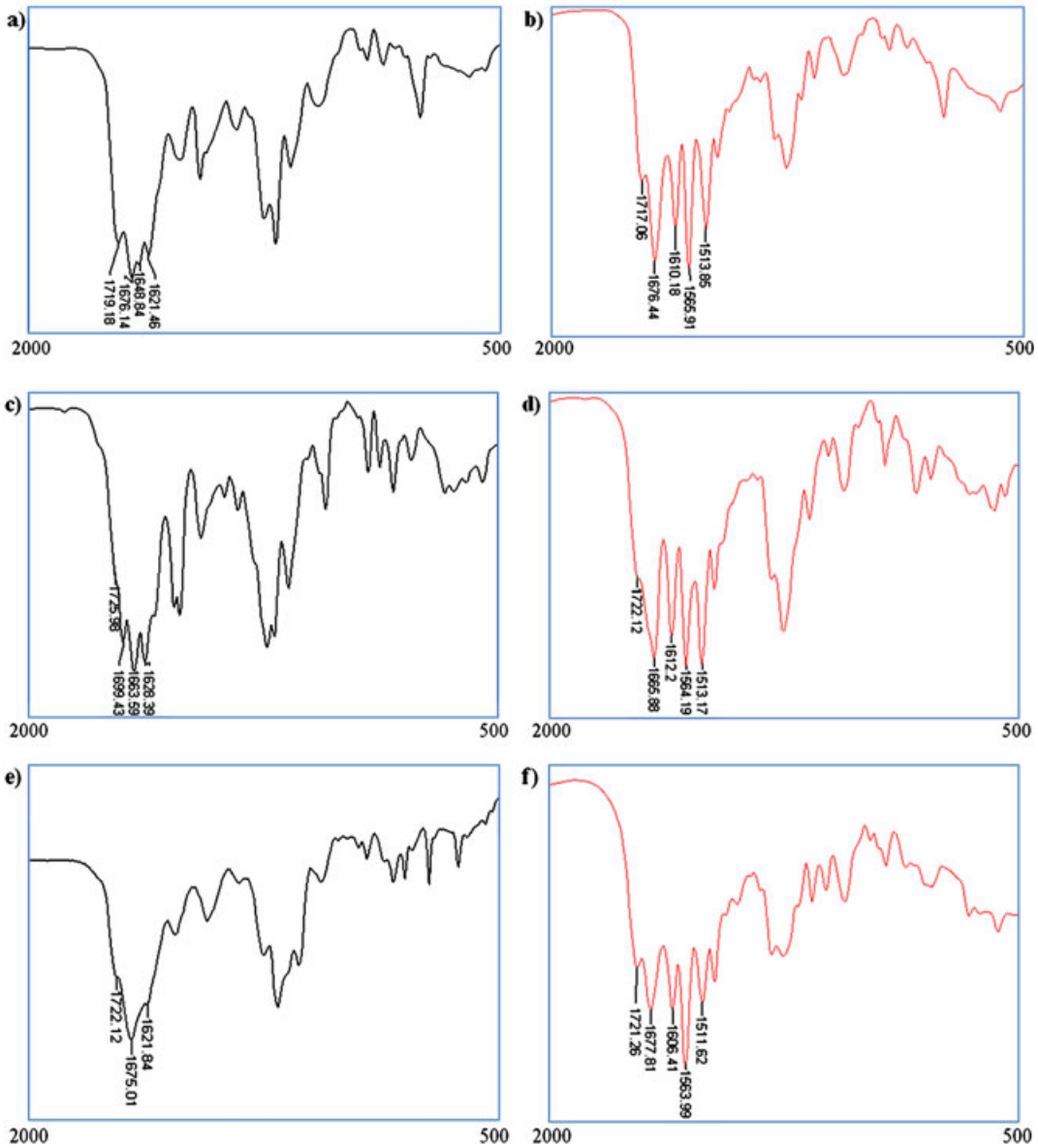

Figure 3. FT-IR spectra of Cu-[KA-AA-NH_2]; (A) KA-W-NH_2; (B) Cu-[KA-W-NH_2]; (C) KA-Y-NH_2; (D) Cu-[KA-Y-NH_2]; (E) KA-H-NH_2; and (F) Cu-[KA-H-NH_2].

Table 1. FT-IR spectra shift of Cu-[KA-AA-NH_2]

	Wavenumber of IR spectra (per cm)				
Compound	Carbamate bond	Amide of C-terminal	Ketone of KA	Newly appeared	
KA-F-NH_2	1720	1671	1617	1513	1567
KA-W-NH_2	1719	1676	1621	1511	1567
KA-Y-NH_2	1725	1663	1628	1513	1564
KA-H-NH_2	1722	1675	1621	1511	1567

Cell Cultures and Measurement of Melanin Contents

The Mel-Ab cell line, which produces large amounts of melanin, is a mouse-derived spontaneously immortalized melanocyte cell line [24]. Mel-Ab cells were cultured in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum, 100 nM 12-*O*-tetradecanoylphorbol-13-acetate (TPA), 1 nM cholera toxin, 50 μg/ml streptomycin and 50 U/ml penicillin at 37 °C in 5% CO_2. The effects of the depigmenting agents on melanin formation in the Mel-Ab cell line were estimated. Cells were treated with 100 μM of the selected copper (II) complexes, Cu-[KA-AA-NH_2],

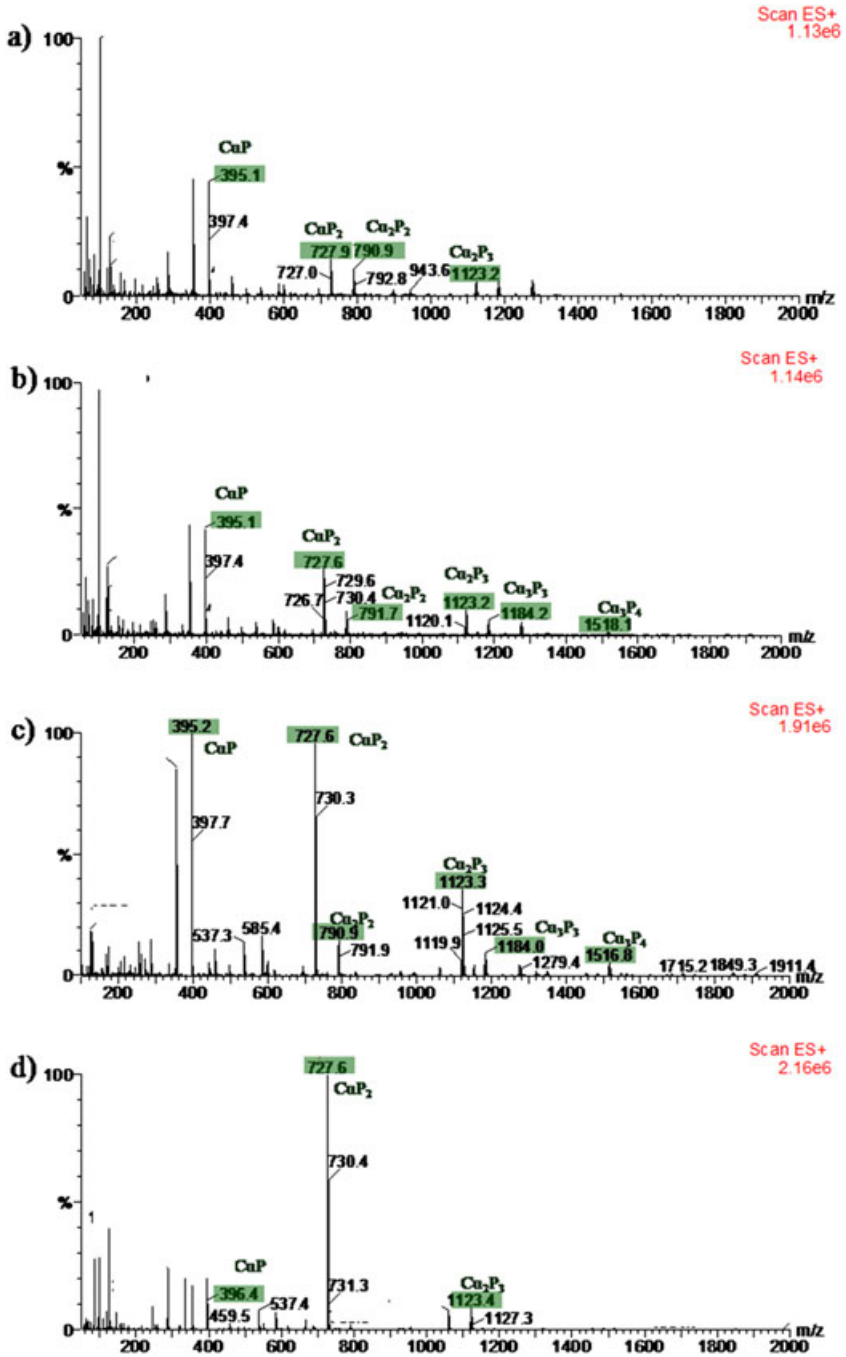

Figure 4. MS of Cu-[KA-F-NH_2]; P means KA-F-NH_2 and Cu is copper; the ratios of P to Cu are (A) 0.5 : 1; (B) 1 : 1; (C) 2 : 1; and (D) 4 : 1.

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for 4 days. The cells were lysed in 1 M NaOH at 100 °C for 30 min and centrifuged for 20 min at 16,000 g. The optical densities of the supernatants were measured at 400 nm. Standard curves of synthetic melanin (0–300 μg/ml) were also prepared in triplicate for each experiment.

Results and Discussion

Preparation of KA-AA-NH_2 Metal Complexes

Initially, kojic acid-7-imidazolide (**2**) was prepared for more effective coupling to amino acids and obtained with a yield of 78%[†] (Figure 1). KA-AA-NH_2 was synthesized using SPPS method (Figure 2) with more than 90% purity. Cu-[KA-F-NH_2] was prepared in various ratios of 4 : 1, 2 : 1, 1 : 1 and 0.5 : 1 (KA-F-NH_2 : Cu). The 1 : 1 ratio had the greatest yield of recovered complex (60% ~ 70%), and other copper complexes were also prepared in this manner. The molar ratio of KA-AA-NH_2 to copper was approximately 1, which was characterized by ICP-atomic emission spectrometer analysis. In contrast, zinc complexes were prepared in KA-AA-NH_2 : Zn = 2 : 1 as the zinc complex did not give an acceptable yield at a ratio of 1 : 1 (Figure 2). In addition, KA-V-NH_2 and KA-FWY-NH_2 copper complexes were prepared to compare their activities as control compounds. After drying *in vacuo*, the metal complexes were obtained as a green powdery form.

FT-IR Spectra of KA-AA-NH_2 Metal Complexes

KA-AA-NH_2 Cu (II) and Zn (II) complexes were directly prepared from KA-AA-NH_2 and soluble metal salts in water or water–methanol mixture. These conversions were measured by FT-IR spectroscopy (Figure 3). The four kinds of Cu-[KA-F-NH_2] complexes formed in different ratio did not show any difference in their IR spectra (data not shown). In the case of compound **2**, the ketone group of KA was detected at 1652/cm, which was shifted to 1760/cm when it became a carbamate bond. The C-terminus amide of phenylalanine amide was detected at 1677/cm. When the carbonyl group participated in complexation with copper (II) or zinc (II) ion, its stretching band shifted towards a lower wave number.

After metal complexation, the absorption peaks of KA-AA-NH_2 showed similar changes in their IR spectrum as KA-F-NH_2 did [22]. New bands appeared at 1513/cm and 1567/cm after metal coordination, which may represent the spectrum of metal coordinated carbonyl groups of KA. However, the bands of carbamate and C-terminal amide bonds were maintained, which means that these carbonyl groups hardly coordinated to the metal ion. Results from the FT-IR spectra are summarized in Table 1.

MS of KA-AA-NH_2 Metal Complexes

In the case of KA-F-NH_2 : Cu = 0.5 : 1, the metal complex was formed incompletely, which is why the peak of Cu-[KA-F-NH_2] had low intensity. When KA-F-NH_2 was reacted to copper in 1 : 1 or 2 : 1 ratio, both peaks of Cu-[KA-F-NH_2] and Cu-[KA-F-NH_2]$_2$ were observed in MS. When KA-F-NH_2 was chelated to copper in 4 : 1 ratio, the peak of Cu-[KA-F-NH_2]$_2$ appeared dominant (Figure 4).

MS results suggested that copper (II) or zinc (II) complexes of KA-AA-NH_2 has different properties from those of KA. As shown in the ESI-MS spectra, in addition to the major signal (CuP and CuP_2), several complex peaks of different stoichiometry were recorded (Figure 5). KA-AA-NH_2 has additional functional groups, although KA coordinates with copper or zinc by a 2 : 1 molar ratio. Therefore, various metal complexes of different ratios are detected in addition to metal complexes of 2 : 1 ratio. This indicates that KA-AA-NH_2 metal complexes might have polymeric structures because of various metal chelating centers, which makes the complexes insoluble in water. However, only one mass peak, which corresponds to the 1 : 1 Cu (II) complex, appeared in the case of KA-H-NH_2 (Figure 5(D)). For complex formation, different solvent systems were used for each KA-AA-NH_2. Because KA-H-NH_2 is very soluble in water, water was used for Cu-[KA-H-NH_2] formation, whereas water–methanol mixture was used for the others because of their limited solubility. To demonstrate the effect of the solvent system on metal complex composition, Cu-[KA-F-NH_2] was prepared in a water-only system, although a much larger reaction volume of five times was required. In this case, the results were similar to Cu-[KA-H-NH_2], with a major peak

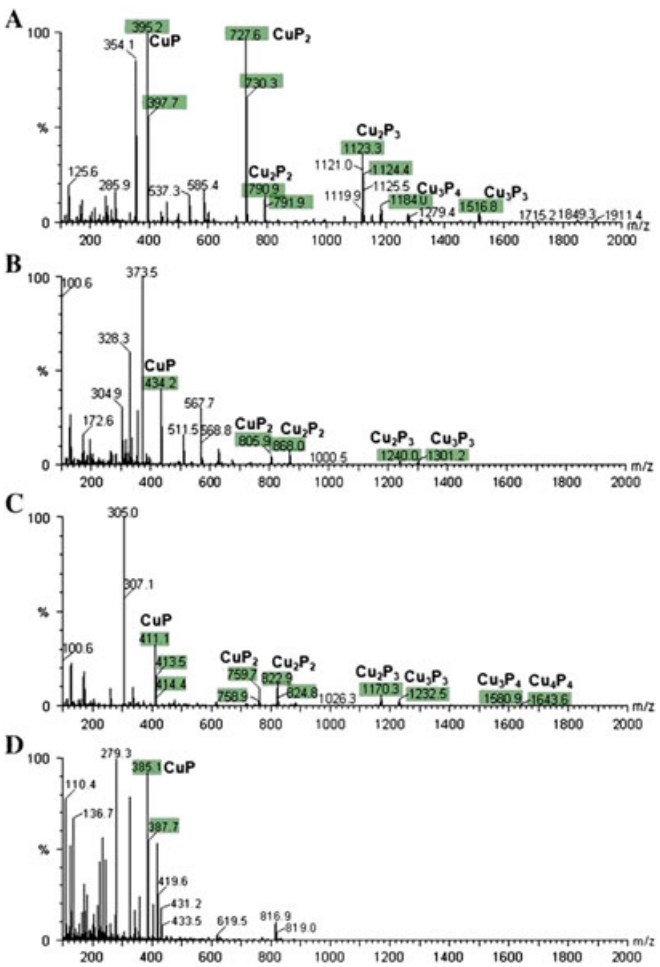

Figure 5. MS of Cu-[KA-AA-NH_2]; (A) Cu-[KA-F-NH_2]; (B) Cu-[KA-W-NH_2]; (C) Cu-[KA-Y-NH_2]; and (D) Cu-[KA-H-NH_2].

[†]The structure of compound **2** was identified by NMR spectra. ^{1}H NMR (300 MHz, DMSO-d6) δ 9.34 (1H, s, –OH), 8.33 (1H, s, N–CH N), 8.13 (1H, s, –CH–O), 7.67 (1H, s, imidazole), 7.10 (1H, s, imidazole), 6.66 (1H, s, CH–C O), 5.30 (2H, s, CH_2–O).

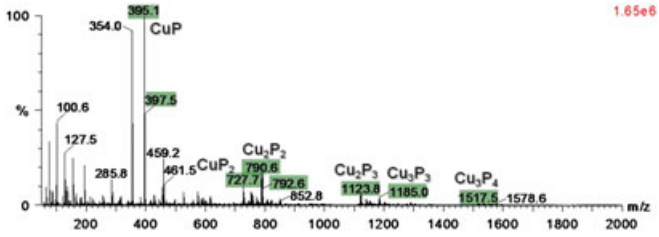

Figure 6. MS of Cu-[KA-F-NH_2] formed in water.

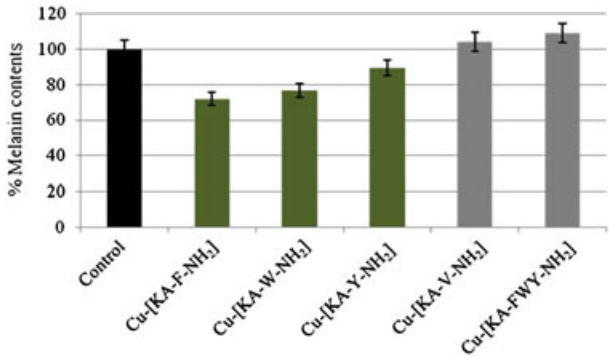

Figure 7. Melanogenesis inhibitory activity in Mel-Ab cells; %Melanin contents were measured after 100 μM of each sample. Black solid bar indicates the result of control sample without any inhibitor. Each experiment was performed in triplicate and averaged.

of the 1 : 1 complex, CuP, appearing in the mass spectra of Cu-[KA-F-NH_2] (Figure 6).

Zn-[KA-AA-NH_2] showed slightly different characteristics to Cu-[KA-AA-NH_2]. Because Zn-O coordination is relatively weaker than that of Cu-O, Zn-[KA-AA-NH_2] did not exhibit a polymeric nature because of extra coordination between KA-AA-NH_2 and zinc (data not shown). Despite KA-Y-NH_2 showing three different stoichiometries such as ZnP, ZnP_2 and Zn_2P_2, it was hard to determine whether the complex was well formed because of difficulties with characterization. The preparation of Zn-[KA-AA-NH_2] is not as easy as that of Cu-[KA-AA-NH_2] probably because of weak Zn-O coordination, and therefore, the recovery yield of Zn-[KA-AA-NH_2] was quite low (10% ~ 30%) compared with Cu-[KA-AA-NH_2] (60% ~ 70%).

Melanogenesis Inhibitory Activity

Although KA-F-NH_2, KA-W-NH_2, KA-Y-NH_2 and KA-FWY-NH_2 had more than 90% of tyrosinase inhibitory activity in the tube test [10,11], they did not reduce melanin formation activity at all in the cell system because of low cell permeability. However, inhibitor-treated cells produced significantly less melanin than the cells without inhibitor treatment. We treated 100 μM of inhibitors to the Mel-Ab cells, and none of the compounds, KA-F-NH_2, KA-W-NH_2, KA-Y-NH_2, KA-V-NH_2, KA-FWY-NH_2 or their copper complexes, showed cytotoxicity at this concentration. Cu-[KA-F-NH_2], Cu-[KA-W-NH_2] and Cu-[KA-Y-NH_2] showed stronger activity than KA, which inhibited melanin synthesis by 9% at 100 μM (Figure 7). We could not measure the activity of Cu-[KA-H-NH_2] because of its low solubility. This result demonstrates that metal coordination helps KA-AA-NH_2 to penetrate the cell membrane by increasing its lipophilicity. The tyrosinase inhibitory activity of KA-V-NH_2 is low, and its copper complex Cu-[KA-V-NH_2] did not reduce melanin content. Cu-[KA-FWY-NH_2] did not show melanogenesis inhibitory activity in the Mel-Ab cells because its large molecular weight may have prevented it from diffusing into the cell.

Conclusion

We prepared copper and zinc complexes using four kinds of KA-AA-NH_2. We demonstrated that not only metal complex of KA-F-NH_2 but also that of various KA-AA-NH_2 worked efficiently on reducing melanin content in melanocytes by increasing cell permeability.

Acknowledgment

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References

1 Kahn V, Ben-Shalom N, Zakin V. Effect of kojic acid on the oxidation of *N*-acetyldopamine by mushroom tyrosinase. *J. Agric. Food. Chem.* 1997; **45**: 4460–4465.
2 Burdock GA, Soni MG, Carabin IG. Evaluation of health aspects of kojic acid in food. *Regul. Toxicol. Pharmacol.* 2001; **33**: 80–101.
3 Kobayashi Y, Kayahara H, Tadasa K, Nakamura T, Tanaka H. Synthesis of amino acid derivatives of kojic acid and their tyrosinase inhibitory activity. *Biosci. Biotechnol. Biochem.* 1995; **59**: 1745–1746.
4 Kadokawa J, Nishikura T, Muraoka R, Tagaya H, Fukuoka N. Synthesis of kojic acid derivatives containing phenolic hydroxy groups. *Synth. Commun.* 2003; **33**: 1081–1086.
5 Rho HS, Baek HS, Ahn SM, Kim MK, Ghimeray AK, Cho DH, Hwang JS. Synthesis and biological evaluation of kojyl thioether derivatives as tyrosinase inhibitors. *Bull. Korean Chem. Soc* 2010; **31**: 2375–2378.
6 Kang SS, Kim HJ, Jin C, Lee YS. Synthesis of tyrosinase inhibitory (4-oxo-4H-pyran-2-yl) acrylic acid ester derivatives. *Bioorg. Med. Chem. Lett.* 2009; **19**: 188–191.
7 Nishimura T, Kometani T, Takii H, Tanaka H, Okada SJ. Purification and some properties of α-amylase from *Bacillus subtilis* X-23 that glucosylates phenolic compounds such as hydroquinone. *Ferment. Bioeng.* 1994; **78**: 37–41.
8 Kobayashi Y, Kayahara H, Tadasa K, Tanaka HH. Synthesis of *N*-kojic-amino acid and *N*-kojic-amino acid-kojiate and their tyrosinase inhibitory activity, *Bioorg. Med. Chem. Lett.* 1996; **6**: 1303–1308.
9 Noh JM, Kwak SY, Kim DH, Lee YS. Kojic acid tripeptide amide as a new tyrosinase inhibitor. *Biopolymers* 2007; **88**: 300–307.
10 Noh JM, Kwak SY, Seo HS, Kim BG, Lee YS. Kojic acid-amino acid conjugates as tyrosinase inhibitors. *Bioorg. Med. Chem. Lett.* 2009; **19**: 5586–5589.
11 Zelphati O, Szoka FC Jr. Intracellular distribution and mechanism of delivery of oligonucleotides mediated by cationic lipids. *Pharm. Res.* 1996; **13**: 1367–1372.
12 Zelphati O, Szoka FC Jr. Mechanism of oligonucleotides release from cationic liposomes. *Proc. Natl. Acad. Sci. U.S.A.* 1996; **93**: 11493–11498.
13 Conradi RA, Hilgers AR, Ho NFH, Burton PS. The influence of peptide structure on transport across Caco-2 cells. *Pharm. Res.* 1991; **8**: 1453–1460.
14 Conradi RA, Hilgers AR, Ho NFH, Burton, PS. The influence of peptide structure on transport across Caco-2 cells, II: peptide bond modification which results in improved permeability. *Pharm. Res.* 1992; **9**: 435–439.
15 Pooga M, Kut C, Kihlmark M, Hallbrink M, Fernaeus S, Raid R, Land T, Hallberg E, Bartfai T, Langel U. Cellular translocation of proteins by transportan. *FASEB J.* 2001; **15**: 1451–1453.
16 Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B, Barsoum J. Tat-mediated delivery of heterologous proteins into cells. *Proc. Natl. Acad. Sci. U.S.A* 1994; **91**: 664–668.
17 Wyman TB, Nicol F, Zelphati O, Scaria PV, Plank C, Szoka FC Jr. Design, synthesis, and characterization of a cationic peptide that binds to nucleic acids and permeabilizes bilayers. *Biochemistry* 1997; **36**: 3008–3017.

18 Fernandez-Carneado J, Kogan MJ, Castel S, Giralt E. Potential peptide carriers: amphipathic proline-rich peptides derived from the N-terminal domain of g-Zein. *Angew. Chem. Int. Ed.* 2004; **43**: 1811–1814.
19 Morris MC, Depollier J, Mery J, Heitz F, Divita G. A peptide carrier for the delivery of biologically active proteins into mammalian cells. *Nat. Biotechnol.* 2001; **19**: 1173–1176.
20 Sheldon K, Liu D, Ferguson J, Gariepy J. Loligomers: design of de novo peptide-based intracellular vehicles. *Proc. Natl. Acad. Sci. U.S.A.* 1995; **92**: 2056–2060.
21 Wender PA, Mitchell DJ, Pattabiraman K, Pelkey ET, Steinman L, Rothbard JB. The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. *Proc. Natl. Acad. Sci. U.S.A.* 2000; **97**: 13003–13008.
22 Kwak SY, Noh JM, Park SH, Byun JW, Choi HR, Park KC, Lee YS. Enhanced cell permeability of kojic acid-phenylalanine amide with metal complex. *Bioorg. Med. Chem. Lett.* 2010; **20**: 738–741.
23 Lee TK, Lee SM, Ryoo SJ, Byun, JW, Lee YS. Application of AM *SURE*™ resin to solid-phase peptide synthesis. *Tetrahedron Lett.* 2005; **46**: 7135–7138.
24 Dooley TP, Gadwood RC, Kilgore K, Thomasco LM. Development of an in vitro primary screen for skin depigmentation and antimelanoma agents. *Skin Pharmacol.* 1994; **7**: 188–200.

