Chemistry and some biological effects of model melanoidins and pigments as Maillard intermediates

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Various pigments were formed in the D-xylose-glycine reaction system. Blue pigments (Blue-M1 and Blue-M2) and red pigments (Red-M1 and Red-M2) were generated in the Maillard reaction. Blue-M2 is presented to have been generated by the reaction between Blue-M1, which involved two pyrrolopyrrole structures as the major blue pigment, and di-D-xyluloseglycine. We identified red pigments as the isomers of addition compounds of D-xyluloseglycine to condensated compound between pyrroropyrrole-2-carbaldehyde and pyrrole-2-carbaldehyde compounds. These pigments have polymerizing activities, suggesting that they are important Maillard reaction intermediates through the formation of melanoidins. Blue-M1 as well as melanoidins effectively suppressed the peroxidation of linoleic acid. The scavenging activity toward Blue-M1 on hydroxyl and DPPH radicals was also as strong as that of melanoidins. Furthermore, Blue-M1 prevents the oxidative cell injury. Therefore, Blue-M1 will be an antioxidant which protects against the oxidative stress in biological systems. Melanoidins induced IFN-γ mRNA and IL-12 mRNA expressions in spleen cells exposed to allergen and in macrophage-like J774.1 cells, respectively. These findings suggest that melanoidins have suppressive effect on allergic reaction as a novel physiological effect.

Keywords: Antioxidant / Advanced glycation end products (AGEs) / Blue pigment / Melanoidins / Pyrroropyrrole

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1 Introduction

Typical nonenzymatic reaction occurring among food components is caused by amino and carbonyl compounds, called amino-carbonyl, carbonyl-amine, nonenzymatic browning reactions, and so on. Maillard reaction originally meant the reaction between amino compounds and reducing sugars but it has recently been used as a synonym for amino-carbonyl reaction. In the Maillard reaction, nonenzymatic glycosylation relevant to proteins is called glycation.

Amino compounds involved in the Maillard reaction in food and biological systems are amines, amino acids, peptides, proteins, base in nucleic acids, phospholipids, and so on. Reducing sugars, aldehydes, ketones, polyphenols, ascorbic acid, steroids, and so on are involved in this reaction as carbonyl sources.

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Abbreviation: AGE, advanced glycation end product

Various phenomena and physiologic effects are caused by the Maillard reaction [1–6]. In food systems, low-molecular weight products contribute to discoloration, the generation of aroma, formation of mutagens, scavenging and generation of reactive oxygen species, and the decrease in nutritional value and digestibility. High-molecular weight products, so-called melanoidins, which are the final products of the Maillard reaction, are brown nitrogen-containing polymeric substances which are difficult to decompose. Human beings daily ingest melanoidins from brown processed foods. Melanoidins demonstrate physiologically positive effects because of unique partial structures in the molecules such as reductones, enamines, and pyrrole-like structure [3–5].

Melanoidins are known to have various physiologically positive effects such as antioxidative activities [7, 8] and strong scavenging activity against reactive oxygen species, *e.g.*, hydroxyl radicals, hydrogen peroxides, and superoxides [1, 2]. It is expected that melanoidins show the physiologic effects *in vivo* in digestive organs because parts of melanoidins were absorbed through the gastrointestinal tracts of rats [9].

Thus, the Maillard reaction in food systems has both desirable and undesirable effects. We should control its effect in processing and storage of foods.

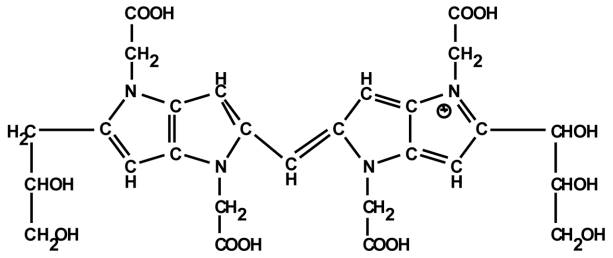

Figure 1. Structure of BlueM1.

Most proteins *in vivo* are nonenzymatically glycated [4, 5]. Reducing sugars can react with amino compounds to form a Schiff base adduct which is then stabilized by an Amadori rearrangement. This reaction represents the early stage of the Maillard reaction. The reaction is followed by the intermediate stage on the generation of α-dicarbonyls such as 3-deoxyosones, osones, methylglyoxal, and glyoxal. Furthermore, in the advanced stage of the Maillard reaction, proteins are modified by advanced glycation end-products (AGEs) with the evolution of fluorescence, browning, polymerization, and degradation.

In biological systems, Maillard reaction is believed to contribute to yellowing and crosslinking of long-lived proteins such as lens crystallins and collagen, and to an array of biochemical and biological abnormalities in diabetes and aging such as stiffening of arteries and joints, increased blood pressure, and altered protein, and cell turnover [10–12]. Such general AGE accumulation is accelerated and linked to arteriosclerosis, nephropathy, neuropathy, retinopathy, and cataract.

The formation mechanisms of melanoidins as AGEs have been left unsolved. Miura and Gomyo [13], Gomyo *et al.* [l4], and the authors [15] have reported the formation of blue pigments in the Maillard reaction between D-xylose and glycine. The blue pigment is postulated to be an intermediate oligomer in the generation of melanoidins. The authors [16] have reported the identification of a novel blue pigment that was designated Blue-M1 (blue Maillard reaction intermediate-1). It consists of four molecules of D-xylose and glycine, and has a methine proton between two pyrrolopyrrole rings as shown in Fig. 1. It is assumed to be a dimer of yellow-colored pyrrolopyrrole-2-carbaldehyde compounds. Blue-M1 has a polymerizing activity, suggesting that it is an important Maillard reaction intermediate through the formation of melanoidins. Blue-M2 and red compounds which have higher molecular weight than that of Blue-M1 were generated in the D-xylose-glycine reaction system. In this review, we identified these pigments which had polymerizing activity, and estimated the formation mechanisms of melanoidins. Nutritional and physiological effects of melanoidins have been widely investigated [5]. In this review, we have studied whether Blue-M1 has antioxidative activity as strong as melanoidins or not. Moreover, we observed the suppressive effects on allergy as a positive physiological effect of melanoidins as AGEs *in vivo* as well as in foods.

2 Materials and methods

2.1 Preparation of melanoidins and identification of the Maillard reaction intermediates

Melanoidins were prepared as described in ref. [17]. D-Glucose (2 M) or D-xylose (2 M), glycine (2 M), and sodium hydrogen carbonate (0.2 M) were dissolved in water. The mixture was refluxed in an oil bath at 95°C for 7 h. The resulting brown solution was dialyzed against water for 2 wk, and the nondialyzable fraction (Glc-Gly MEL and Xyl-Gly MEL) was then lyophilized. Xyl-BuNH_2 MEL was also obtained from the reaction mixture of D-xylose and *n*-butylamine neutralized with acetic acid in methanol at 50°C for 7 days [18].

Blue-M2, Red-M1, and Red-M2 were isolated from the reaction mixture of D-xylose (1 M) and glycine (0.1 M) dissolved in 60% ethanol (starting pH 8.1) under nitrogen and stored at 26.5°C for 48 h before being stored at 2°C for 96 h. These pigments were purified by DEAE-Sephadex A-25 column, Bio-Gel-P-2 column, and RP-HPLC according to the elution conditions described in a previous paper [16, 19]. Each solution was then put on a Sephadex G-15 column to complete the desalting process, respectively. The chemical structures of these purified pigments were determined based on ^{13}C-NMR and ^{1}H-NMR (500 MHz), FAB-MS data, and MALDI and TOF mass spectra (TOF-MS) [16].

2.2 Generation and measurement of hydroxyl and DPPH radicals

Generation and measurement of reactive oxygen species were performed as previously described [20]. Briefly, DMPO (5, 5-dimethylpyrroline-*N*-oxide) was used as a spin-trapping reagent for ESR analysis. Hydroxyl radicals were generated from a Fenton reaction. Ten microliters of 3% H_2O_2, 10 μL of 250 mM DMPO, and 10 μL of sample in 0.2 M phosphate buffer at pH 7.4 were mixed, and added 10 μL of 5 mM $FeSO_4$. The mixture was moved in borosilicate glass capillary column. The DMPO-OH adducts were measured by ESR spectrometer JEX-RE1X for 5 min after the addition of $FeSO_4$.

Similarly, DPPH radicals were measured. DPPH (7.92 mg) was dissolved in 10 mL of ethanol. Two hundred microliters of DPPH and 10 μL of samples in phosphate buffer at

pH 7.4 were mixed, and then after 5 min, the DPPH radicals were measured by ESR.

Mn^{2+} was used as an internal standard to calculate the relative amounts from ESR signal intensity. Measurement of peroxide value was done as in a previous paper [20].

2.3 Preparation of methylated Blue-M1

Blue-M1 was methylated as previously described [20]. Briefly, Blue-M1 (0.5 mg) was dissolved in 0.8 mL of methanol and 2.8 mL of benzene. To the solution was added 200 μL of trimethylsilyl-diazomethane, and incubated at room temperature for 30 min. The presence of methylated Blue-M1 in this solution was confirmed by HPLC and MS. HPLC was done by monitoring at 625 nm under the following conditions: RP-HPLC with a solvent system of 0.005 M tetrabutyl ammonium phosphate in water/methanol (60:40), using a Mightysil RP-18 (Kanto Chemical, 250 mm × 4.6 mm id). TOF-MS was recorded with a Voyager RP mass spectrometer (PE Biosystems). The ionization mode was set to MALDI at an accelerating voltage of 20 kV with 2, 5-dihydroxybenzoic acid as a matrix.

2.4 Effects of Blue-M1 against cytotoxicity on COS-1 cells

We assessed the protective effect of Blue-M1 against AAPH-induced cytotoxicity on COS-1 cells [21]. Cell viability was measured with tetrazolium salt, WST-8. COS-1 cells were cultured with DMEM supplemented with 10% fetal bovine serum, 10 U/mL penicillin, and 10 μg/mL streptomycin and kept in a humidified atmosphere containing 5% CO_2 at 37°C. Cells were washed twice with PBS, and suspended in medium at an initial density of 1×10^5 cells/mL. Each 100 μL of cell suspension was seeded in a 96-well culture plate, and cultured at 37°C for 24 h. Then, COS-1 cells were exposed in 50 μL of Blue-M1 (0.02–0.2%)-containing medium, and then 50 μL of 30 mM AAPH was added. Cells were cultured at 37°C for 24 h, and washed twice with PBS. Cells were exposed in 100 μL of medium and 10 μL of WST-8 solution was added. Cells were incubated at 37°C for 1 h, and reduction of WST-8 was monitored at 450 nm with a multilabel counter. Lipid peroxide of cell membrane was detected with dipheny-1-pyrenylphosphine (DPPP) reagent.

2.5 Suppressive effect on allergy reaction by melanoidins

Suppressive effects on allergic reaction were assessed on mouse spleen cells exposed with ovalbumin and on macrophage-like mouse J774.1 cells [19]. Cells were cultured and treated with melanoidins which were prepared from the reaction mixture of 6-aminocaproic acid (1 M), glucose (1 M), and sodium hydrogen carbonate (0.2 M) refluxed for 4 h. RNA was extracted and expression of IFN-γ, IL-4, and IL-12 p40 mRNA was measured by RT-PCR.

3 Results and discussion

3.1 Blue-M1 from D-xylose and glycine

When D-xylose and glycine was reacted at 26.5°C, the reaction mixture produced yellow, red, and blue pigments [13–15]. The isolated blue pigment (Blue-M1, $C_{27}H_{31}N_4O_{13}$) revealed its novel chemical structure as shown in Fig. 1 [16]. Blue-M1 had two pyrrolopyrrole rings coupled with methine bridge. Figure 2 is a proposed formation pathway to Blue-M1. Two yellow pigments (Yellow-1 and -2) would be formed by condensation between N-substituted pyrrole-2-carbaldehyde and Schiff base of glycine and 3-deoxyxylosone or xylosone. Blue-M1 was considered to be formed by coupling of Yellow-1 and -2. In total, Blue-M1 would be formed from 4 mol Amadori compounds through 9 mol dehydration, 1 mol dehydrogenation, and 1 mol decarboxylation. As a result, Blue-M1 kept 5 residual hydroxyl groups belonging to parent xylose moiety (Fig. 1).

Blue-M1 could be produced under low temperature, and was observed to be able to polymerize through interaction with their yellow pigments. We propose that Blue-M1 is a key intermediate for melanoidin formation.

3.2 Melanoidin-1 from D-xylose and butylamine

Butylamine was used as a model for lysine residue of protein. The nondialyzable melanoidin preparation, melanoidin-1, which had an elemental composition of $C_{10.9}H_{15.6}N_1O_{2.7}$, consumed periodate, and was susceptible to acetylation and tritylation, indicating that some –CHOH–CH_2OH groups remained [22]. When melanoidin-1 was pyrolyzed at 600°C, major pyrolyzates were *N*-butylpyrrole and *N*-butyl-2-methylpyrrole, indicating the presence of pyrrole ring skeleton in the melanoidin [18, 22].

3.3 Comparison of Blue-M1 with melanoidin-1

Chemical composition of both Blue-M1 and melanoidin-1 was shown in Table 1, which was calculated from experimental data and/or theoretical ones. Higher sugar moiety and lower hydroxyl content in melanoidin-1 would be responsible to involvement of nonnitrogeneous products such as furfural. Dehydration ratio of furfural, however, is

Table 1. Comparison of chemical composition of Blue-M1 and melanoidin-1[a)]

	Sugar moiety *per* nitrogen[b)]	Residual –OH content (mol)	Periodate consumption	Dehydration ratio (xH_2O)[b)]	Dehydrogenation ratio (xH_2)[b)]
Blue-M1	1.0	1.25	0.75[d)]	2.25	0.25
Melanoidin-1	1.2	0.71[c)]	0.84	2.3	0.4

a) All values were calculated as those *per* sugar moiety of parent Amadori compounds.
b) Calculated from elemental composition.
c) Calculated from acetyl group content of acetylated melanoidin-1.
d) Theoretical value.

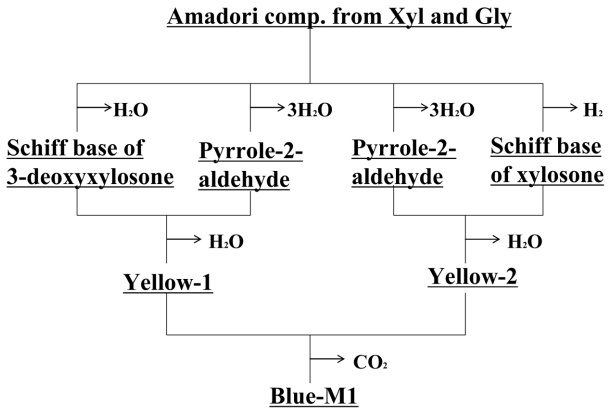

Figure 2. Proposed formation pathway of Blue-M1.

similar to that of pyrrole-2-carbaldehyde. Dehydrogenation occurred also in melanoidin-1 as well as Blue-M1. Table 1 seems to indicate some resemblance between Blue-M1 and melanoidin-1.

3.4 Possibility of the presence of pyrrolopyrrole in AGEs

Fluorescent and yellow or brown AGEs are considered to be formed mainly from D-glucose and proteins. According to the proposed pathway shown in Fig. 2, N-substituted pyrrole-2-carbaldehyde condenses with Schiff base of 2-ketoaldehyde to form a pyrrolopyrrole ring. We have already reported that D-glucose-butylamine reaction system produced smaller amounts of *N*-butylpyrrole-2-carbaldehyde in addition to its 5-hydroxymethyl derivative (pyrraline) [23]. If D-glucose and proteins could produce *N*-substituted pyrrole-2-carbaldehyde, there would be a possibility of formation of pyrrolopyrrole in colored AGEs.

3.5 Identification of Maillard reaction pigments

Blue-M2 was isolated and purified by RP-HPLC. UV-Vis and fluorescent spectra of Blue-M2 showed maximum peak at 643 nm; and Ex_{max}, 350 nm and Em_{max}, 445 nm. FAB-MS for Blue-M2 showed the presence of the M^+ ion at *m/z* 940. The high-resolution FAB-MS data for Blue-M2 showed as follows. *m/z* (M^+): Calcd. for $C_{39}H_{50}O_{22}N_5$: 940.2948, Found 940.2943. Blue-M2 was identified as the addition compound of di-D-xyluloseglycine to Blue-M1 which involved two pyrrolopyrrole structures by NMR spectra as shown in Fig. 3 [19]. MALDI-TOF-MS data (a, b, c, d, and e fragments) by PSD mode (MS/MS) for a parent ion (Da = 940) of Blue-M2 supported the identified structure as shown in Fig. 3.

In addition, red pigments were also formed in the D-xyloseglycine reaction system. UV-Vis of Red-M1 and Red-M2

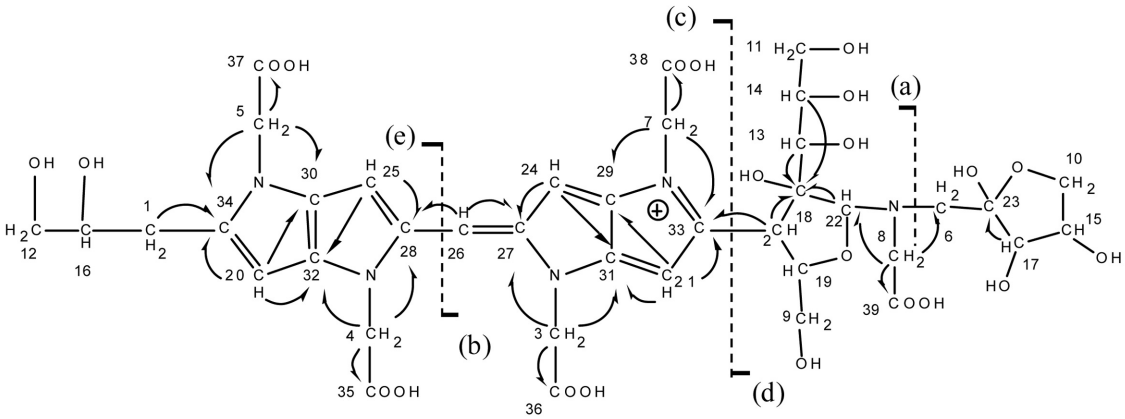

Figure 3. Structure of Blue-M2.

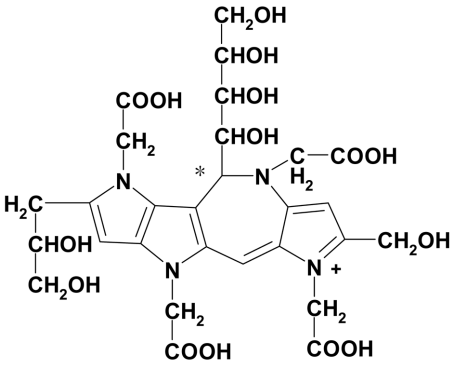

Figure 4. Structure of Red-M1 and Red M2. * Assymmetric carbon for isomer.

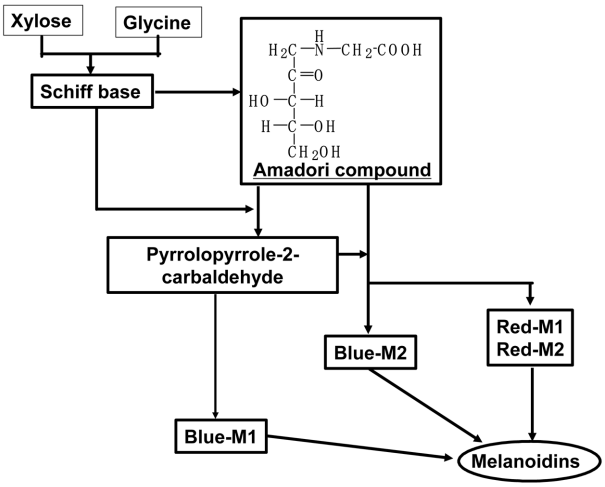

Figure 5. Proposed formation mechanisms of pigments and melanoidins.

showed maximum peak at 564 and 554.5 nm, respectively. Both FAB-MS data for Red-M1 and Red-M2 showed the presence of the M^+ ion at *m/z* 667. The high-resolution FAB-MS data for Red-M1 and Red-M2 showed as follows. *m/z* (M^+): Calcd. for $C_{28}H_{35}O_{15}N_4$: 667.2099, Found 667.2068 (Red-M1) and 667.2151 (Red-M2). We identified red pigments (Red-M1 and Red-M2) as isomers of addition compounds of D-xyluloseglycine to condensated compound between pyrroropyrrole-2-carbaldehyde and pyrrole-2-carbaldehyde compounds as shown in Fig. 4 [19]. The structures were confirmed by PSD mode (MS/MS) of the MALDI-TOF-MS for each parent ion (Da = 667) of Red-M1 and Red-M2.

These blue and red pigments have polymerizing activity, suggesting that they are important Maillard reaction intermediates through the formation of melanoidins. Blue-M1 (Fig.1) was not formed from Amadori compound (D-xyluloseglycine) but the generation of Blue-M1 was increased by the addition of D-xyluloseglycine to the D-xylose-glycine reaction system. Blue-M1 might be generated by the decarboxylation from two molecules of yellow pigments, possibly two pyrrolopyrrole-2-carbaldehyde compounds [15]. Identification of the precursor of Blue-M1 is now in progress. Moreover, D-xyluloseglycine was prepared from xylose and glycine deuterated on methylene group. Added the D-xyluloseglycine was incorporated in a part of di-D-xyluloseglycine of Blue-M2 structure in the xylose-glycine reaction system. Accordingly, Blue-M2 is supposed to be generated from di-D-xyluloseglycine and two pyrrolopyrrole-2-carbaldehyde compounds as shown in Fig. 5. Red-M1 and Red-M2 might be generated from D-xyluloseglycine, pyrroropyrrole-2-carbaldehyde, and pyrrole-2-carbaldehyde. Moreover, Blue-M1, Blue-M2, and Red compounds are assumed to be generated independently and polymerized to be formed melanoidins.

3.6 Antioxidative activity of pigments

Figure 6 shows the antioxidative activity of BHA, α-tocopherol, the melanoidins, and Blue-M1. The antioxidative activity was calculated as follows: POV% = POV of the test sample/POV of control × 100. POV of the linoleic acid control solution incubated at 50°C for 24 h was 106.2 and POV% of Blue-M1 at a concentration of 230 μM (0.014%) was 35 [20]. Therefore, 65% suppression of lipid peroxidation was observed in Blue-M1 compared with the control. POV% of the melanoidins (0.025%) prepared from Glc-Gly and Xyl-Gly reaction systems were 22 and 30, respectively. POV% of BHA and α-tocopherol were 5.3 and 22, respectively. These results indicate that Blue-M1 has antioxidative activity as strong as that of the melanoidins and α-tocopherol.

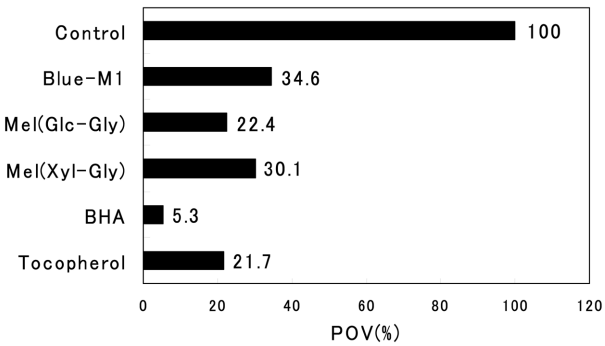

Figure 6. Effect of Blue-M1 and melanoidins, BHA, α-tocopherol on the peroxide value of linoleic acid.

3.7 Scavenging activity of reactive oxygen species

Figure 7 shows the relative ESR spectra of DMPO spin adducts with hydroxyl radicals in the presence of Blue-M1, the melanoidins, BHA, and α-tocopherol. These samples

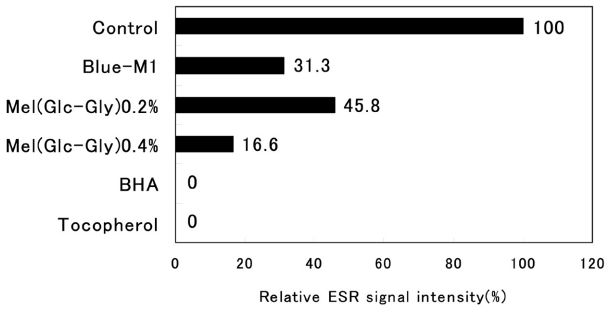

Figure 7. Scavenging activity of Blue-M1, BHA, and α-tocopherol against hydroxyl radicals.

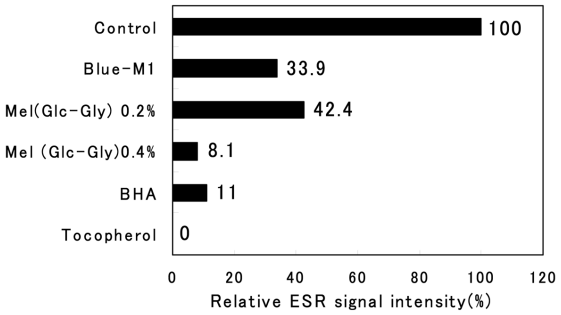

Figure 8. Scavenging activity of Blue-M1, BHA, and α-tocopherol against DPPH radicals.

scavenged effectively hydroxyl radicals [20]. Therefore, Blue-M1 had hydroxyl radical-scavenging activity as strong as that of the melanoidins.

Hydroxyl radicals are well known to react readily with various organic compounds such as proteins, carbohydrates, and lipids. The authors [17, 24] have previously shown melanoidins at concentrations of 0.3 and 0.03%, respectively, scavenged 86 and 47% of the hydroxyl radicals generated by 10 kGy of ^{60}Co γ-rays. However, fructose and mannitol are known scavengers of hydroxyl radicals at a concentration of 0.5% scavenged 20 and 0%, respectively. The higher hydroxyl radical-scavenging activity by melanoidins than by known scavengers may depend on the unique partial structure, excepting the hydroxyl groups. Blue-M1 is composed of D-xylose and glycine. Sugars have scavenging activity against hydroxyl radicals. However, scavenging activity of sugars is not so strong as melanoidins [17, 20, 24]. Therefore, we speculate that the strong scavenging activity of Blue-M1 was not due to hydroxyl groups in the sugar moiety, but other structures.

The authors [25] have shown that glycated protein also scavenged the hydroxyl radicals formed by γ-irradiation (10 kGy). The scavenging ability of glycated proteins against hydroxyl radicals is considered to depend on Maillard reaction products in the advanced stage, such as N^{ε}-lysyl-5-hydroxymethylpyrrole-2-carbaldehyde called pyrraline and melanoidins.

Our findings revealed that the blue pigment changed to yellow color after the reaction of Blue-M1 and hydroxyl radicals. We suggest that long conjugated system expressing the blue color may have been broken by hydroxyl radicals. Accordingly, we speculate that hydroxyl radical-scavenging activity by Blue-M1 might have been due to the pyrrolopyrrole ring and methine bridge.

Figure 8 shows the relative ESR signal intensity of the DPPH radicals with each sample. BHA and α-tocopherol strongly scavenged DPPH radicals. Blue-M1 at a concentration of 3 mM (0.19%) scavenged 66% of the DPPH radicals, while 0.2% melanoidins scavenged 58% of the DPPH radicals. Blue-M1 had as strong scavenging activity toward DPPH radicals as that of melanoidins. The scavenging activity of Blue-M1 might have been dependent on the protons in the pyrrolopyrrole ring or methine proton. An NMR experiment revealed that these protons were readily reactive in Blue-M1 and yellow pigments ([20]; Hayase, F. *et al*., unpublished results). Murakami *et al*. [26] have reported that the radical-scavenging activity in the early stage of the Maillard reaction between D-xylose and glycine was derived from uncolored reaction products smaller than the brightly colored blue pigments. It would be very interesting to identify the uncolored reaction products. However, both Blue-M1 and Blue-M2, which could have been formed by the addition reaction of the yellow pigment to Blue-M1 showed fairly strong scavenging activity against DPPH radicals (Hayase, F. *et al*., unpublished results). The oxidation-accelerating substances might have existed in the frac-

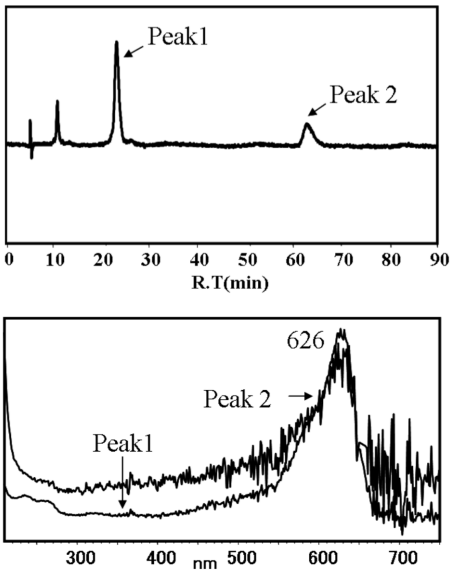

Figure 9. HPLC pattern and UV-Vis spectra of methylated Blue-M1 and Blue-M1. Peak 1: methylated Blue-M1, peak 2: Blue-M1.

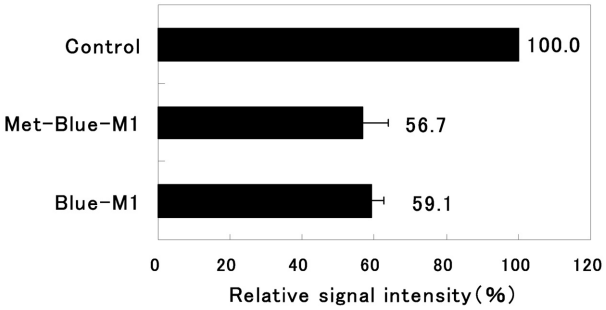

Figure 10. Hydroxyl radical-scavenging activity of methylated Blue-M1. The concentration of Blue-M1 was 0.16 mM.

tion containing blue colored pigments that was separated by Murakami *et al* [26].

Melanoidins have been proposed to have scavenging activity against reactive oxygen species due to their pyrrole-like and reductone structures, and chelating ability against transition metals due to their anionic structures [2]. Methylated Blue-M1 was derived from Blue-M1 (MW = 619) in order to investigate the effects of four carboxyl groups on the generation of antioxidative activity. The HPLC pattern of methylated Blue-M1 indicates that peak 1 was methylated Blue-M1 as shown in Fig. 9. These peaks have a maximum absorbance at 626 nm. The MALDI-TOF-MS data measured in the linear mode for methylated Blue-M1 showed the presence of the M^+ ion at m/z = 674.97. These results indicate that Blue-M1 was completely methylated.

Figure 10 shows the scavenging activity against hydroxyl radicals of methylated Blue-M1 in comparison with that of Blue-M1. The results indicate strong scavenging activity of methylated Blue-M1 like that of Blue-M1. It is considered that the four carboxyl groups in Blue-M1 did not take part in the scavenging activity against hydroxyl radicals by Blue-M1. We speculate that the four carboxyl groups do not have strong chelating activity.

Blue-M1 was incubated with H_2O_2 for 3 h in order to determine oxidation product of Blue-M1 by peroxidation. Figure 11 shows NMR spectrum of Blue-M1 incubated without H_2O_2 (A) and with H_2O_2 (B). Peaks a′, b′, c′, d′, and e′ were observed after the addition of H_2O_2. The oxidation product is supposed to be involved an addition structure of hydroperoxide on methine carbon of Blue-M1 as indicated in Fig.11.

Consequently, pyrrolopyrrole ring and a methine bridge between two pyrrolopyrrole rings could be related the appearance of the radical-scavenging activity.

3.8 Effects of Blue-M1 against cytotoxicity on COS-1 cells

Blue-M1, Blue-M2, Red-M1, and Red-M2 showed strong antioxidative activities as well as melanoidins [20]. COS-1 cells were cultured in AAPH containing DMEM medium with or without Blue-M1 at 37°C for 24 h. Blue-M1

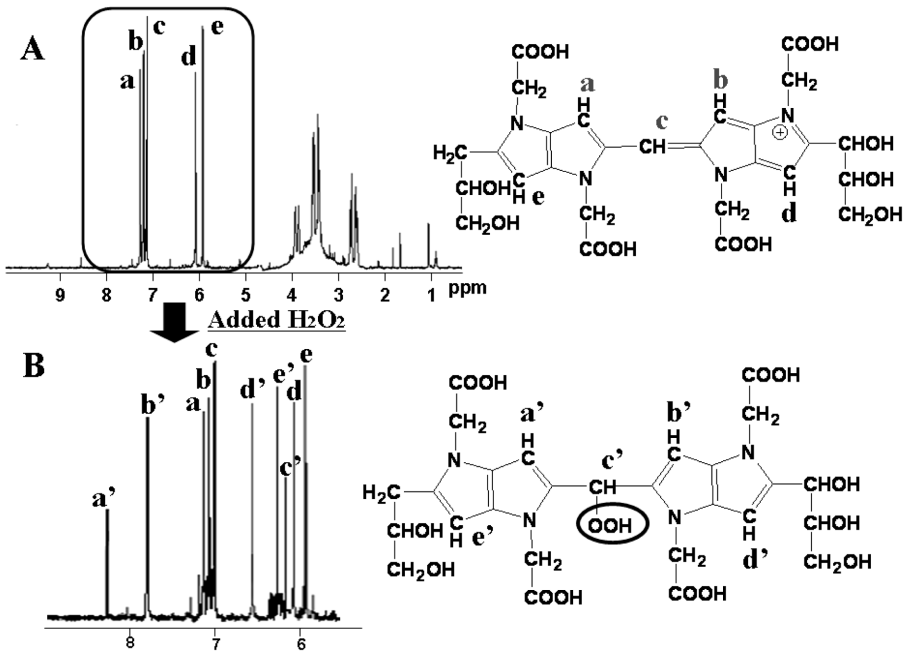

Figure 11. NMR spectrum of Blue-M1 incubated without H_2O_2 (A) and with H_2O_2 for 3 h (B).

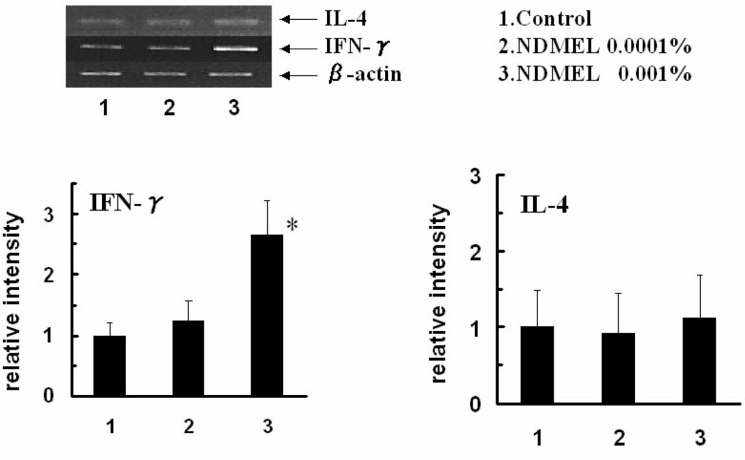

Figure 12. Effect of melanoidins on IFN-γ and IL-4 mRNA expressions in splenocytes. Values are mean ± SD. $n = 3$, $^*p < 0.05$ *vs.* control.

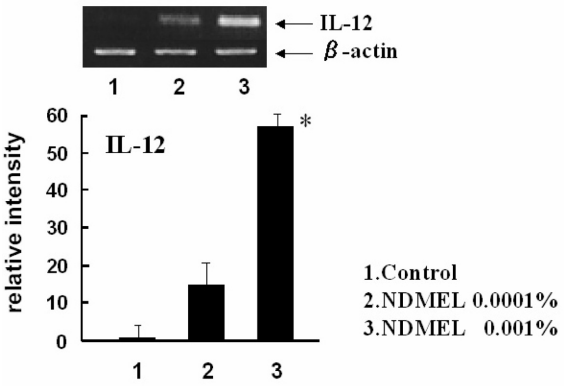

Figure 13. Effect of melanoidins on IL-12 p40 mRNA expression in J774. 1. Values are mean ± SD. $n = 3$, $^*p < 0.05$ *vs.* control.

decreased the AAPH-induced toxicity in COS-1 cells, and this effect was dose dependent. Furthermore, COS-1 cells were treated with DPPP, as a reagent for the detection of lipid peroxide, and then were cultured in AAPH containing DMEM medium with or without Blue-M1 at 37°C for 6 h. Blue-M1 prevented the AAPH-induced peroxidation of cell membrane on COS-1 cells, and this effect was also dose dependent. These results suggest that Blue-M1 prevents the oxidative cell injury. Therefore, Blue-M1 will be an antioxidant, which protects against the oxidative stress in biological systems [21].

3.9 Suppresive effect on allergic reaction by melanoidins

Figure 12 indicates that the melanoidins-induced expression of IFN-γ mRNA but not IL-4 mRNA in spleen cells exposed to ovalbumin as allergen. Figure 13 shows the induction of IL-12 p40 mRNA expression by melanoidins in macrophages like J774.1 cells. These findings suggest that the melanoidins have suppressive effect on allergic reaction as a novel physiological effect.

4 Concluding remarks

Blue-M1, Blue-M2, Red-M1, and Red-M2, which are blue and red pigments generated from the D-xylose-glycine reaction system, are useful as model compounds to estimate the major structure of melanoidins and colored AGEs. Blue-M1 has a strong antioxidative activitiy as well as melanoidins. Melanoidins have a suppressive effect on allergic reaction as a novel physiological effect. However, more research is needed to better understand the formation mechanism of melanoidins through intermediate pigments, and physiological effects of melanoidins *in vivo*.

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5 References

[1] Hayase, F., Kato, H., *Comm. Agric. Food Chem*. 1994, *3*, 111 – 128.

[2] Hayase, F., in: Ikan R. (Ed.), *The Maillard Reaction*, John Wiley and Sons, England 1996, pp. 89 – 104.

[3] Friedman, M., *J. Agric. Food Chem*. 1996, *44*, 631 – 653.

[4] Horiuchi, S., Taniguchi, N., Hayase, F., Kurata, T., Osawa, T. (Eds.), *The Maillard Reaction in Food Chemistry and Medical Science: Update for The Postgenomic Era*, Elsevier, Amsterdam 2002, pp. 1 – 527.

[5] Baynes, J. W., Monnier, V. M., Ames, J. M., Thorpe, S. R. (Eds.), *The Maillard Reaction-Chemistry at the Interface of Nutrition, Aging, and Disease*, Ann. N. Y. Acad. Sci. 2005, *1043*, pp. 1 – 954.

[6] Lee, T.-C., Chichester, C. O., in: Finley, J. W., Schwass, D. E. (Eds.), *Xenobiotics in Foods and Feeds*, ASC Symposium Series 234, 1983, pp. 379 – 408.

[7] Kim, S. B., Hayase, F., Kato, H., *Dev. Food. Sci.* 1986, *13*, 383 – 392.

[8] Yamaguchi, N., *Dev. Food. Sci.* 1986, *13*, 291 – 299.

[9] Lee, I. E., Chuyen, N. V., Hayase, F., Kato, H., *Biosci. Biotech. Biochem*. 1992, *56*, 21 – 23.

[10] Ledl, F., Schleicher, E., *Angew. Chem. Int. Ed. Engl*. 1990, *29*, 565 – 594.

[11] Monnier, V. M., Sell, D. R., Nagaraj, R. H., Miyata, S., *Gerontology* 1991, *37*, 152 – 165.

[12] Cerami, A., in: Labuza, T. P., Reineccius, G. A., Monnier, V. M., O'Brien, J. *et al.* (Eds.), *Maillard Reactions in Chemistry, Food, and Health*, The Royal Society of Chemistry, Cambridge 1994, pp. 1 – 10.

[13] Miura, M., Gomyo, T., *Nippon Nougeikagaku Kaishi (in Japanese)* 1982, *56*, 417 – 425.

[14] Gomyo, T., Haiyan, L., Miura, M., Hayase, F., Kato, H., *Agric. Food Chem*. 1989, *53*, 949 – 957.

[15] Hayase, F., *Food Sci. Technol. Res*. 2000, *6*, 79 – 86.

[16] Hayase, F., Takahashi, Y., Tominaga, M., Miura, T. *et al.*, *Biosci. Biotech. Biochem*. 1999, *63*, 1512 – 1514.

[17] Hayase, F., Hirashima, S., Okamoto, G., Kato, H., *Agric. Biol. Chem*. 1989, *53*, 3383 – 3385.

[18] Hayase, F., Sato, M., Tsuchida, H., Kato, H., *Agric. Biol. Chem*. 1982, *46*, 2987 – 2996.

[19] Hayase, F., Usui, T., Nishiyama, K., Sasaki, S. *et al.*, in: Baynes, J. W., Monnier, V. M., Ames, J. M., Thorpe, S. R. (Eds.), *The Maillard Reaction-Chemistry at the Interface of Nutrition, Aging, and Disease*, *Ann. N. Y. Acad. Asci*. 2005, *1043*, pp. 104 – 110.

[20] Shizuuchi, S., Hayase, F., *Biosci. Biotechnol. Biochem*. 2003, *67*, 54 – 59.

[21] Usui, T., Shizuuchi, S., Watanabe, H., Hayase, H., *Biosci. Biotechnol. Biochem*. 2004, *68*, 247 – 249.

[22] Kato, H., Tsuchida, H., in: Eriksson C. (Ed.), *Maillard Reactions in Foods, Prog. Fd. Nutr. Sci.*, Pergamon Press, Oxford 1981, *5*, pp. 147 – 156.

[23] Hayase, F., Kato, H., *Agric. Biol. Chem.* 1985, *49*, 467 – 473.

[24] Hayase, F., Hirashima, S., Okamoto, G., Kato, H., in: Fiont, P. A., Aeschbacher, H. U., Hurrell, R. F., Liardon, R. (Eds.), *The Maillared Reaction in Food Processing, Human Nutrition and Physiology*, Birkhauser Verlag, Basel 1990, pp. 361 – 366.

[25] Okamoto, G., Hayase, F., Kato, H., *Biosci. Biotech. Biochem.* 1992, *56*, 928 – 931.

[26] Murakami, M., Shigeeda, A., Danjo, K., Yamaguchi, T., Matoba, T., *J. Food Sci*. 2002, *67*, 93 – 96.

