Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1411–1417

BIOORGANIC & MEDICINAL CHEMISTRY

Oxidative Deamination of Benzylamine by Glycoxidation

Mitsugu Akagawa, Takeshi Sasaki and Kyozo Suyama*

Department of Applied Bioorganic Chemistry, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, Sendai, 981-8555, Japan

Received 15 October 2002; accepted 14 November 2002

Abstract—In the present study, model reactions for the oxidative deamination by glycoxidation using benzylamine were undertaken to elucidate the detail of the reaction. Glucose, 3-deoxyglucosone (3-DG), and methylglyoxal (MG) oxidatively deaminated benzylamine to benzaldehyde in the presence of Cu^{2+} at a physiological pH and temperature but not glyoxal. 3-DG and MG were more effective oxidants than glucose. We have determined the effects of metal ions, pH, oxygen, and radical scavengers on the oxidative deamination. The formation of benzaldehyde was greatest with Cu^{2+}, and was accelerated at a higher pH and in the presence of oxygen. EDTA, catalase, and dimethyl sulfoxide significantly inhibited the oxidation, suggesting the participation of reactive oxygen species. From these results, we propose a mechanism for the oxidative deamination by the Strecker-type reaction and the reactive oxygen species-mediated oxidation during glycoxidation.

Introduction

The nonenzymatic glycation reaction (Maillard reaction) is thought to contribute to the pathogenesis of diabetic complications[1–6] and the aging process.[1,7–10] In this reaction, free amino groups of protein react slowly with the carbonyl groups of reducing sugars to yield Schiff base intermediates, which then undergo Amadori rearrangement to yeild stable ketoamine derivatives (Amadori products). Subsequently, the Schiff bases and Amadori products degrade into α-dicarbonyl such as 3-deoxyglucosone (3-DG), methylglyoxal (MG), and glyoxal (GO).[11] These compounds are more reactive than the parent sugars with respect to their ability to react with amino groups of proteins. These α-dicarbonyl compounds irreversibly modify lysine and arginine residues in proteins at physiological conditions, leading to the formation of various advanced glycation end products (AGEs) which frequently have chromophores, fluorophores, and protein cross-links.[1,3–6,12,13] Recent researches have demonstrated that the formation and accumulation of AGEs in plasma and tissue proteins are associated with aging[1,7–10] and the long-term complications of diabetes.[1–6] Therefore, it has been recently proposed that α-dicarbonyl stress is among the major factors in the pathogenesis of diabetic complications. Moreover, glycation can give rise to oxygen free radicals in the presence of O_2 and transition metal ions,[14–17] and the formation of some AGEs has been shown to require oxygen free radicals.[17–19] The production of oxidants by the oxidation of glucose-protein adducts has been termed glycoxidation.

Recently, we found that the level of α-aminoadipic-δ-semialdehyde, which is an oxidative deamination product of lysine residue, in rat plasma protein is significantly higher in streptozotocin-induced diabetic rats compared with normal controls. Furthermore, we demonstrated that the lysine residue of bovine serum albumin (BSA) is oxidatively deaminated during the incubation with various carbohydrates in the presence of Cu^{2+} at a physiological pH and temperature.[20] This experiment showed that 3-DG and MG are the most efficient oxidants of the lysine residue. When the reaction was initiated from glucose, a significant amount of α-aminoadipic-δ-semialdehyde was also formed in the presence of Cu^{2+}. The reaction was significantly inhibited by deoxygenation, catalase, and a hydroxyl radical scavenger, suggesting the participation of the reactive oxygen species in the reaction. From these results, we

Abbreviations: BSA, bovine serum albumin; 3-DG, 3-deoxyglucosone; DMSO, dimethyl sulfoxide; EDTA, ethylendiaminetetraacetic; MG, methylglyoxal; GO, glyoxal; HPLC, high performance liquid chromatography.

*Corresponding author. Tel.: +81-22-717-8818; fax: +81-22-717-8820; e-mail: suyama@bios.tohoku.ac.jp

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00584-9

proposed the mechanism for the oxidative deamination by the α-dicarbonyl-mediated Strecker-type reaction and the reactive oxygen species-mediated oxidation during glycoxidation. Furthermore, we hypothesized that the formation of the highly reactive α-aminoadipic-δ-semialdehyde is involved in the development of diabetic complications.

In this research, to characterize the oxidative deamination by glycoxidation, we carried out a model reaction using benzylamine under a *quasi*-physiological condition. A model glycoxidation between benzylamine and carbohydrates in the presence of Cu^{2+} produced significant amount of benzaldehyde. To determine the participation of reactive oxygen species, we also investigated the effect of radical scavengers. On the basis of these data sets, we made a mechanistic interpretation.

Results

The oxidative deamination by glycoxidation was assessed by the reaction of benzylamine with glucose, 3-DG, MG, and GO. Benzylamine (5.0 mM) was incubated in phosphate buffer (50 mM) with each carbohydrate (1.0 mM) under a physiological pH and temperature (pH 7.4, 37 °C). The time course of the oxidative deamination of benzylamine was monitored by the formation of benzaldehyde. As shown in Figure 1A, MG oxidatively deaminated benzylamine to benzaldehyde but not glucose, 3-DG, or GO. There have been various investigations of transition metal ions showing the stimulation of the formation of AGEs[1,21] and α-dicarbonyls[11] in vitro. We examined the effect on the oxidation by incubation of benzylamine with each carbohydrate in the presence of Cu^{2+} . As shown in Figure 1B, in the presence of 250 mM Cu^{2+}, a significant amount of aldehyde was produced by the reaction with glucose, 3-DG, and MG. There was an increase in the concentration of benzaldehyde over the incubation period (14 days). Nevertheless, GO produced only a small amount of benzaldehyde. The final formation of benzaldehyde by glucose, 3-DG, MG, and GO after 14 days was 11.5±0.2 μM, 31.3±1.1 μM, 48.6±2.1, and 0.7±0.1 μM (mean±SEM, $n=3$), respectively.

Because the oxidation was apparently stimulated by the addition of Cu^{2+}, we investigated the effects of physiologically important metals on the reaction. Benzylamine (5.0 mM) was incubated with 1.0 mM glucose, 3-DG, or MG in the presence of each metal ion (250 mM) at pH 7.4 and 37 °C for 7 days. As shown in Fig. 2, the formation of benzaldehyde by glucose, 3-DG, and MG was the greatest with Cu^{2+}. MG significantly oxidized benzylamine irrespective of the presence or absence of each metal ion. 3-DG also underwent the oxidation in the presence of VO^{2+} and Ag^{+}. Nevertheless, only Cu^{2+} catalyzed the oxidation by glucose. No metal ions other than Cu^{2+} catalyzed the oxidation by GO (data not shown).

The effect of Cu^{2+} concentration on the oxidative deamination by glucose, 3-DG, and MG was also explored. Figure 3 shows the results obtained when a fixed amount of benzylamine (5.0 mM) and glucose, 3-DG, or MG (1.0 mM) were incubated with various concentrations of Cu^{2+} (0, 125, 250, and 500 mM) for 7 days. The formation of benzaldehyde was apparently dependent on the concentration of Cu^{2+}.

To find the other factors governing the formation of benzaldehyde, we examined the effect of pH on the oxidative deamination. Reaction mixtures with pH values varying from 6.0 to 10.0, containing benzylamine (5.0 mM), $CuSO_4$ (250 μM), and glucose, 3-DG, or MG (1.0 mM) were incubated at 37 °C for 7 days. As shown in Figure 4, benzaldehyde was formed even faster in basic solutions. Nevertheless, at pH 6, the oxidation was

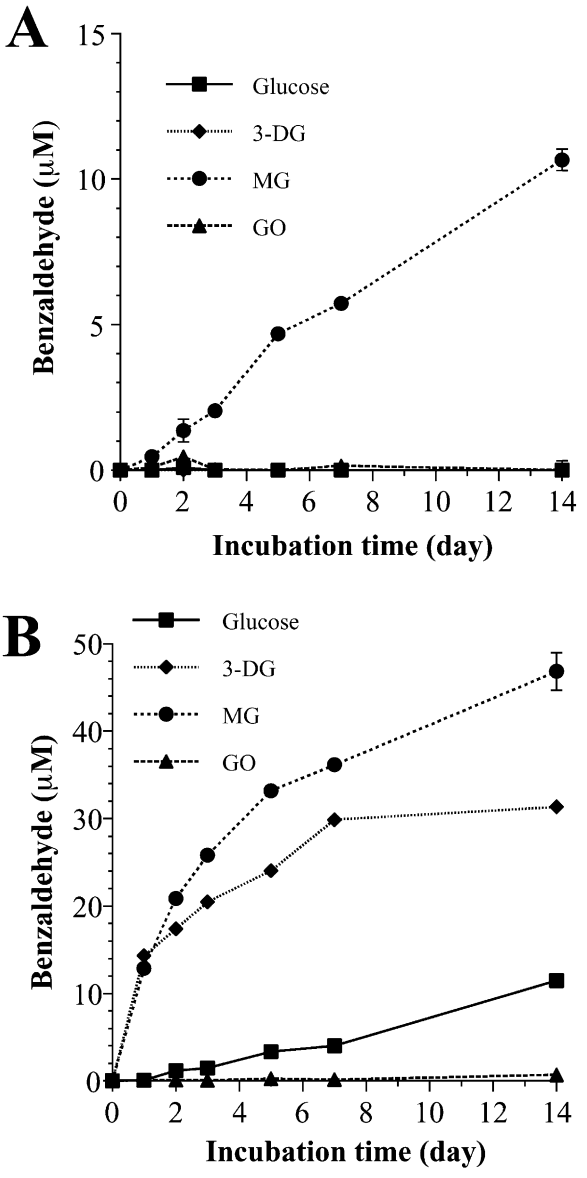

Figure 1. Time course of oxidative deamination of benzylamine by glucose, 3-DG, and MG. Benzylamine (5.0 mM) was incubated with 1.0 mM of each carbohydrate in 50 mM phosphate buffer (pH 7.4) in the presence or absence of Cu^{2+} (250 μM) at 37 °C. After the reaction was terminated, benzaldehyde was measured by HPLC. *A*, without Cu^{2+}. *B*, with Cu^{2+} . The values are shown as means±SEM ($n=3$).

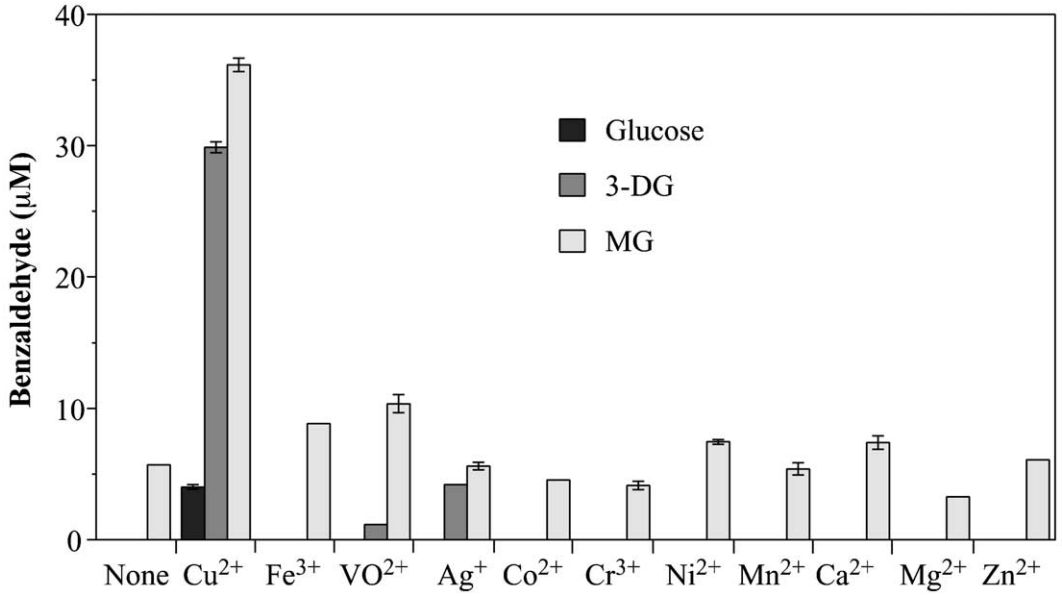

Figure 2. Effect of various kinds of metal ions on oxidative deamination of benzylamine by glucose, 3-DG, and MG. Benzylamine (5.0 mM) was incubated with 1.0 mM of each carbohydrate in 50 mM phosphate buffer (pH 7.4) in the presence or absence of indicated metal ions (250 μM) at 37 °C for 7 days. After the reaction was terminated, benzaldehyde was measured by HPLC. The values are shown as means ± SEM ($n = 3$).

hardly observed in the reaction with glucose and 3-DG. Interestingly, at pH 10, glucose produced the greatest amount of benzaldehyde.

The presence of oxygen is important in the Maillard reaction,[21] and, actually, oxygen is required for the formation of some AGEs.[17,22] To assess the role of oxygen in the reaction, we incubated benzylamine with glucose, 3-DG, and MG in the presence of Cu^{2+} under a nitrogen atmosphere. Indeed, the reaction under a nitrogen atmosphere markedly inhibited the production of benzaldehyde, clearly illustrating the involvement of oxygen in the oxidative deamination (Table 1).

The possibility was considered that one-electron-reduced oxygen intermediates may be generated in the reaction, possibly including hydrogen peroxide, superoxide, and hydroxyl radical which in turn might contribute to the oxidative reaction. Actually, reactive oxygen species have been reported to be generated in the early and the advanced glycation processes,[14–16] and these species have been shown to participate in the for-

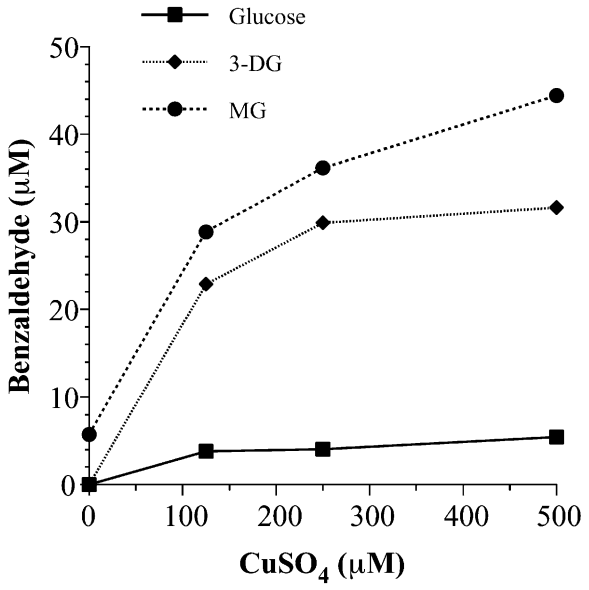

Figure 3. Oxidative deamination of benzylamine by glucose, 3-DG, and MG as a function of Cu^{2+} concentration. Benzylamine (5.0 mM) was incubated with 1.0 mM of each carbohydrate in 50 mM phosphate buffer in the presence or absence of Cu^{2+} (0–500 μM) at 37 °C for 7 days. After the reaction was terminated, benzaldehyde was measured by HPLC. The values are shown as means ± SEM ($n = 3$).

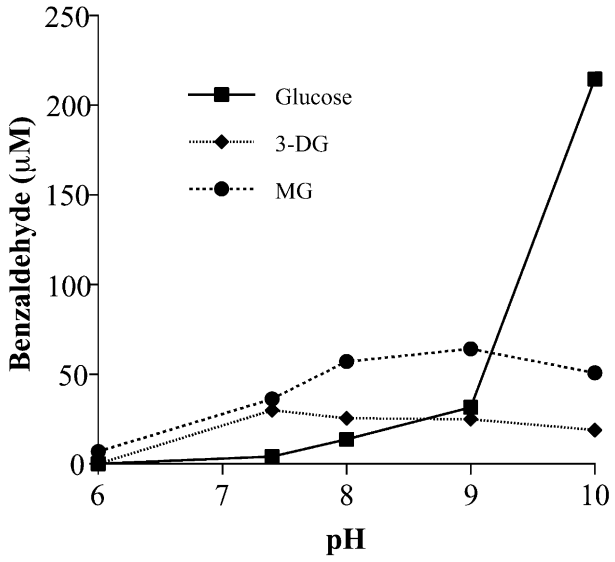

Figure 4. Effect of pH on oxidative deamination of benzylamine by glucose, 3-DG, and MG. Benzylamine (5.0 mM) was incubated with 1.0 mM of each carbohydrate in 50 mM phosphate buffer (pH 6–10) in the presence of Cu^{2+} (250 μM) at 37 °C for 7 days. After the reaction was terminated, benzaldehyde was measured by HPLC. The values are shown as means ± SEM ($n = 3$).

mation of some AGEs.[17–19] Furthermore, we have recently demonstrated that various primary amines, including the lysine residue of BSA, are oxidatively deaminated to the corresponding aldehyde in the Fenton-type system.[23] Thus, benzylamine and Cu^{2+} were incubated with 1.0 mM of glucose, 3-DG, MG, and H_2O_2 in the presence or absence of catalase, a radical scavenger, and a metal ion chelator. As shown in Table 1, addition of catalase (100 U/mL) completely inhibited the benzaldehyde formation by glucose and H_2O_2 and significantly inhibited it by 3-DG and MG. The oxidation of benzylamine by glucose, 3-DG, and MG was also significantly inhibited in the presence of 50 mM dimethyl sulfoxide (DMSO) which is a hydroxyl radical scavenger. Furthermore, marked inhibition was observed in the incubation with H_2O_2 in the presence of DMSO (71.8% inhibition). Addition of a copper chelator (250 mM), ethylenediaminetetraacetic acid (EDTA), effectively inhibited the oxidation by glucose, 3-DG, and MG as well as H_2O_2 (78.7–100% inhibition). These results suggest that the hydroxyl radical is produced by the Fenton-type reaction and is responsible for the benzaldehyde formation.

Discussion

In a previous paper, we reported that glycoxidation caused the oxidative deamination of the lysine residue in BSA and that 3-DG and MG were the most efficient oxidants.[20] In that study, we characterized the long-term process of the glycoxidative modification of BSA.[20] This report shows that the reaction is likewise caused in a short incubation time, offering for the first direct proof of glycoxidation-induced oxidative deamination. In the present study, model experiments using benzylamine were undertaken to elucidate the details of the oxidative deamination reaction by glycoxidation. Measurements of benzaldehyde allowed us to define further the characteristics and mechanism of the reaction. 3-DG and MG oxidatively deaminated benzylamine to benzaldehyde at a physiological pH and temperature in the presence of Cu^{2+}. When the reaction was initiated from glucose, a significant amount of benzaldehyde was also formed in the presence of Cu^{2+}, probably due to the generation of α-dicarbonyls. We have determined the effects of metal ions, pH, oxygen, and radical scavengers on the oxidative deamination. The formation of benzaldehyde by glucose, 3-DG, and MG was greatest with Cu^{2+}, and the oxidation was accelerated at a higher pH, and required oxygen. Furthermore, EDTA, catalase, and DMSO significantly inhibited the oxidation of benzylamine. These data are consistent with our previous results using BSA.[20] From these results, once again we propose that the oxidative deamination during glycoxidation is caused by the α-dicarbonyls-mediated Strecker-type reaction and reactive oxygen species as shown in Figure 5.

Table 1. Effect of N_2, EDTA, and scavengers on the oxidative deamination of benzylamine by carbohydrates

Condition	% Inhibition			
	Glucose	3-DG	MG	H_2O_2
N_2[a]	60±1.5	83.7±0.2	60.8±1.4	—
Catalase (100 U/mL)[b]	100.0±0.0	68.9±6.7	24.8±4.1	100.0±0.0
DMSO (50 mM)	33.2±1.3	29.1±0.7	22.5±4.4	71.8±1.4
EDTA (250 μM)	100.0±0.0	85.5±1.5	78.7±0.0	81.4±0.3

Benzylamine (5.0 mM) was incubated with each carbohydrate (1.0 mM) or H_2O_2 (1.0 mM) in 50 mM phosphate buffer (pH 7.4) in the presence of Cu^{2+} (250 μM) and indicated materials at 37 °C for 7 days. After the reaction was terminated, benzaldehyde was measured by HPLC. The values are shown as means±SEM ($n=3$).

[a]Incubation under N_2 atmosphere.

[b]In the control experiment, benzylamine (5.0 mM) was incubated with each carbohydrate (1.0 mM) or H_2O_2 (1.0 mM) in 50 mM phosphate buffer (pH 7.4) in the presence of Cu^{2+} (250 μM) and heat-inactivated catalase at 37 °C for 7 days.

The decarboxylation/deamination of amino acids to carbonyls is known as the so-called Strecker degradation in food science. In the Strecker degradation,[24,25] a number of carbohydrate-derived α-dicarbonyls as well as glucose are able to degrade α-amino acids at high temperatures, thus generating an aldehyde with one carbon atom less than α-amino acids. On the other hand, *o*-quinone compounds, which have an α-dicarbonyl group, are known to catalyze the oxidative deamination of primary amines to form the corresponding aldehydes under physiological conditions.[26,27] Recent researches have shown that α-dicarbonyls are formed in early glycation from the degradation of glucose and Schiff base adducts in the presence of trace metal ions.[11] Therefore, the α-dicarbonyls derived from glycoxidation potentially may serve as adventitious oxidants of amines and, in the course of the oxidative deamination, generate aldehydes. In practice, a model experiment showed that 3-DG and MG are more efficient oxidants of benzylamine than glucose. Although GO produced only a small amount of benzaldehyde, assuming its reactivity, GO may preferentially form stable cross-links or carboxymethyllysine type compounds during the incubation with benzylamine.[28,29] On the basis of these facts, Figure 5 shows the proposed mechanism of the formation of benzaldehyde from benzylamine by the Strecker-type reaction. The formation of α-dicarbonyls is induced through the autoxidation of glucose and the degradation of Amadori products or Schiff bases adduct by metal ion-catalysis.[11] Subsequently, the resulting MG and 3-DG could condense with benzylamine to form a Schiff base adduct, an iminoketone (I). Then the α-proton of benzylamine would be abstracted by basic media, and the enolization might give an iminoenaminol (**II**). In the electron transfer process, it is assumed that Cu^{2+} serves as the electron-pair acceptor and stabilized **II** through the formation of a coordination complex because the requirement of Cu^{2+} was observed in model studies. Finally, spontaneous hydrolysis of **II** can lead to the release of an enaminol (**III**) and the formation of benzaldehyde. The mechanism of the Strecker-type reaction is not accompanied by decarboxylation and is consistent with the *o*-quinone-mediated mechanism previously proposed.[27] The action of transition metal ions in this mechanism is in agreement with the experimental observation that the oxidative deamination by glucose required transition metal ions.

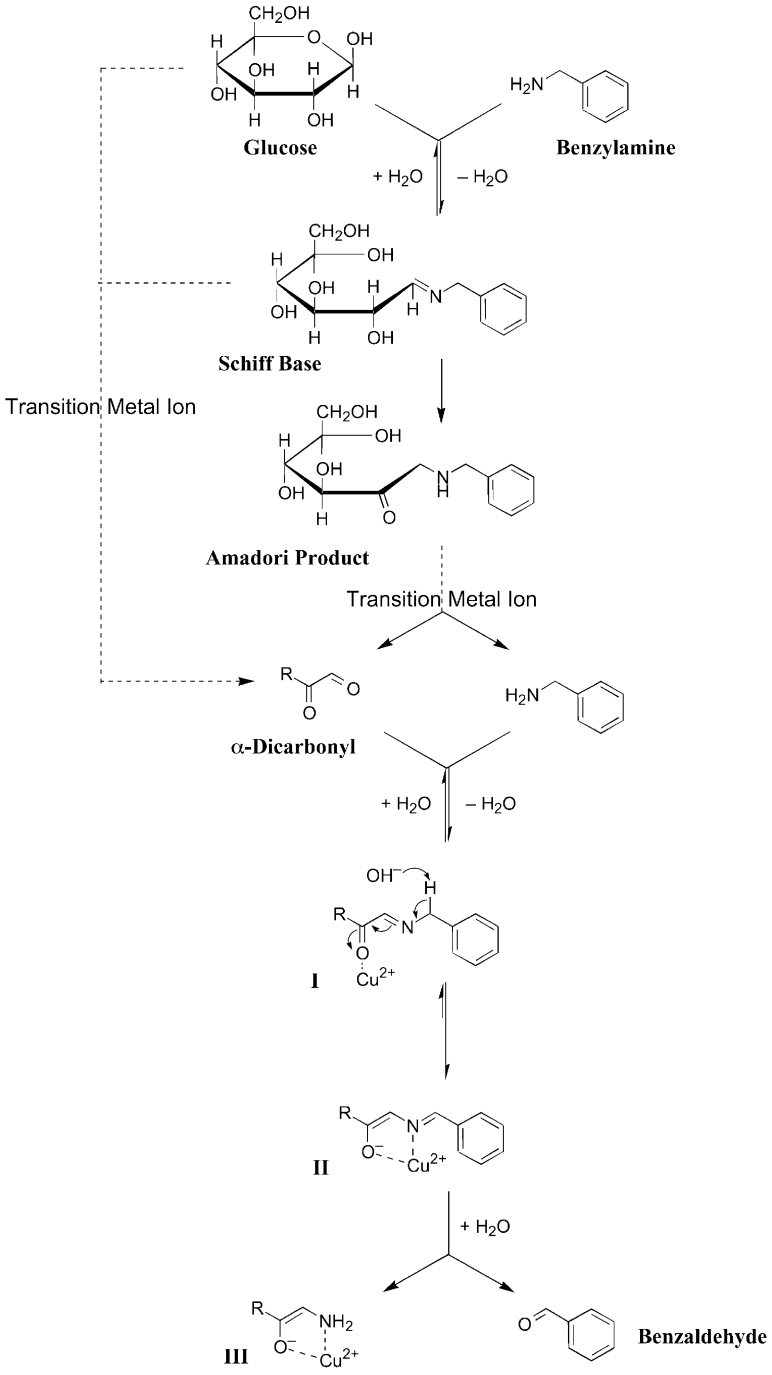

Figure 5. Proposed mechanism of oxidative deamination of lysine residue by the Strecker-type reaction.

Another possible pathway of the oxidative deamination by glycoxidation is reactive oxygen species-mediated oxidative deamination. The fact that the aerobic glycation in the presence of transition metals is accompanied by radical-generating reactions supports this possibility.[14] Furthermore, 3-DG, MG, and GO also produce superoxides during the reaction with lysine and 10 arginine.[16] In the model reaction, catalase and DMSO significantly inhibited the oxidation of benzylamine to benzaldehyde by glucose, 3-DG, and MG, indicating the participation of hydroxyl radicals. The dependency of the oxidative deamination on both oxygen and Cu^{2+} is also consistent with a metal ion-catalyzed mechanism for the production of hydroxyl radicals, probably through the intermediacy of superoxide and H_2O_2. Recently it has been demonstrated that lysine residue is oxidatively deaminated to α-aminoadipic-δ-semialdehyde residue by reactive oxygen species.[23,30,31] In addition, we have found that various primary amines are converted to the corresponding aldehydes in the presence of H_2O_2 and transition metal ions, and the oxidation is effectively prevented by catalase, DMSO, and EDTA.[23] Therefore, the hydroxyl radical generated by the Fenton-type reaction is also likely to contribute to the oxidative deamination by glycoxidation.

The results presented here suggest that the oxidative deamination is one of the major reactions in glycoxidation. Once generated, the aldehydes can condense with each other through aldol condensation or with other amines through Schiff base formation, leading to formation of the complex products. Therefore, the oxidative deamination may play an important part in the formation of AGEs by glycoxidation.

Conclusion

The data presented above clearly demonstrate that glycoxidation induces the oxidative deamination. Glucose, 3-DG, and MG oxidatively deaminated benzylamine to benzaldehyde in the presence of Cu^{2+} at a physiological pH and temperature. 3-DG and MG were much effective oxidants compared with glucose. Deoxygenation and radical scavengers significantly inhibited the reaction. From these results, we propose the mechanism for the oxidative deamination by the Strecker-type reaction and the reactive oxygen species-mediated oxidation during glycoxidation.

Experimental Materials

Acetonitrile was of HPLC grade from Nacalai Tesque Co., Kyoto, Japan. Catalase from bovine liver was from Tokyo Kasei Co., Tokyo, Japan. 3-DG was from Dojindo Laboratories Co., Kumamoto, Japan. All other chemicals were from Nacalai Tesque Co.

Detection of benzaldehyde in incubation of benzylamine with glucose, 3-DG, MG, and GO

General procedure. Reaction mixtures (100 μL) in a micro test tube contained 10 mM benzylamine, 500 mM CuSO4, and 100 mM sodium phosphate buffer, pH 7.4. Typical reactions were started by the addition of 2.0 mM glucose, 3-DG, MG, and GO (100 μL). The reaction mixtures were incubated at 37 °C with shaking in the dark. After incubation, the reaction was terminated by the addition of acetic acid (100 μL). We confirmed that this procedure completely stopped the reaction. After centrifugation at 7740 *g* for 5 min at room temperature, the samples (20-μL aliquots) were chromatographed with acetonitrile/distilled water (4:6, v/v) containing 0.1% phosphoric acid. High performance liquid chromatography (HPLC) was performed on a Perkin Elmer Liquid Chromatograph Integral 4000 system (Norwalk, CT, USA) using a reverse-phase HPLC column (Cosmosil 5C18-AR- II, 150×4.6 mm, Nacalai Tesque Co.) with detection at 245 nm. The column oven was maintained at 30 °C. Benzaldehyde was eluted at 5.2 min at a flow rate of 1.0 mL/min.

Effect of metal ions. Reaction mixtures (100 mL) in a micro test tube contained 10 mM benzylamine, 500 mM of each metal ion ($CuSO_4$, $VOSO_4$, $MnCL_2$, $AgNO_3$, $CoCl_2$, $FeCl_3$, $CiCl_2$, $ZnCl_2$, $CrCl_3$, $MgCl_2$, and $CaCl_2$) and 100 mM sodium phosphate buffer, pH 7.4. The reaction was started by the addition of 2.0 mM glucose, 3-DG, MG, and GO (100 μL). The reaction mixtures were incubated at 37 °C with shaking in the dark. After incubation for 7 days, the reaction was terminated by the addition of acetic acid (100 μL). Then the production of benzaldehyde was measured by HPLC as described above.

Effect of pH. Reaction mixtures (100 μL) in a micro test tube contained 13 mM benzylamine, 500 μM $CuSO_4$ and 100 mM sodium phosphate buffer at various pH values (6, 7.4, 8, 9, and 10). The reaction was started by the addition of 2.0 mM glucose, 3-DG, and MG (100 μL). The reaction mixtures were incubated at 37 °C with shaking in the dark. After incubation for 7 days, the reaction was terminated by the addition of acetic acid (100 mL). Then the production of benzaldehyde was measured by HPLC as described above.

Incubation under nitrogen. Reaction mixtures (100 μL) in a Pyrex test tube contained 10 mM benzylamine, 500 μM $CuSO_4$, and 100 μM sodium phosphate buffer (pH 7.4). After the addition of 2.0 mM glucose, 3-DG, and MG, the test tube was tightly fitted with a silicone rubber cap. The tube was immediately evacuated and then filled with N_2 gas through a hypodermic needle. After another hypodermic needle was inserted in the tube to serve as an outlet port, gas was passed through the incubation mixture for 10 min and charged until the pressure of 0.05 MPa inside the tube was reached. Then the reaction mixture was incubated at 37 °C for 7 days with shaking in the dark.

Effect of catalase. Reaction mixtures (100 μL) in a micro test tube contained 10 mM benzylamine, 500 μM $CuSO_4$, and 100 mM sodium phosphate buffer (pH 7.4) in the presence of catalase (200 U/mL) or heat-inactivated catalase (200 U/mL at 100 °C for 20 min). The reactions were started by the addition of 2.0 mM glucose, 3-DG, and MG (100 μL). The reaction mixtures were incubated at 37 °C with shaking in the dark. After incubation, the reaction was terminated by the addition of acetic acid (100 mL).

References and Notes

1. Singh, R.; Barden, A.; Mori, T.; Beilin, L. *Diabetologia* **2001**, *44*, 129.
2. Obayashi, H.; Nakano, K.; Shigeta, H.; Yamaguchi, M.; Yoshimori, K.; Fukui, M.; Fujii, M.; Kitagawa, Y.; Nakamura, N.; Nakamura, K.; Nakazawa, Y.; Ienaga, K.; Ohta, M.; Nishimura, M.; Fukui, I.; Kondo, M. *Biochem. Biophys. Res. Commun.* **1996**, *226*, 37.
3. Chellan, P.; Nagaraj, R. H. *Arch. Biochem. Biophys.* **1999**, *368*, 98.
4. Hayase, F.; Nagaraj, R. H.; Miyata, S.; Njoroge, F. G.; Monnier, V. M. *J. Biol. Chem.* **1989**, *263*, 3758.
5. Sady, C.; Jiang, C. L.; Chellan, P.; Madhun, Z.; Duve, Y.; Glomb, M. A.; Nagaraj, R. H. *Biochim. Biophys. Acta* **2000**, *1481*, 255.
6. Wilker, S. C.; Chellan, P.; Arnold, B. M.; Nagaraj, R. H. *Anal. Biochem.* **2001**, *290*, 353.
7. Verzijl, N.; DeGroot, J.; Oldehinkel, E.; Bank, R. A.; Thorpe, S. R.; Baynes, J. W.; Bayliss, M. T.; Bijlsma, J. W. J.; Lafeber, F. P. J. G.; TeKoppele, J. M. *Biochem. J.* **2000**, *350*, 381.

8. Verzijl, N.; DeGroot, J.; Thorpe, S. R.; Bank, R. A.; Shaw, J. N.; Lyons, T. J.; Bijlsma, J. W. J.; Lafeber, F. P. J. G.; Baynes, J. W.; TeKoppele, J. M. *J. Biol. Chem.* **2000**, *275*, 39027.
9. Bailey, A. J. *Mechan. Age. Develop.* **2001**, *122*, 735.
10. Frye, E. B.; Degenhardt, T. P.; Thorpe, S. R.; Baynes, J. W. *J. Biol. Chem.* **1998**, *273*, 18714.
11. Thornalley, P. J.; Langborg, A.; Minhas, H. S. *Biochem. J.* **1999**, *344*, 109.
12. Glomb, M. A.; Monnier, V. M. *J. Biol. Chem.* **1995**, *270*, 10017.
13. Oya, T.; Hattori, N.; Mizuno, Y.; Miyata, S.; Maeda, S.; Osawa, T.; Uchida, K. *J. Biol. Chem.* **1999**, *274*, 18492.
14. Wolff, S. P.; Jiang, Z. Y.; Hunt, J. V. *Free Radic. Biol. Med.* **1991**, *10*, 339.
15. Hunt, J. V.; Bottoms, M. A.; Mitchinson, M. J. *Biochem. J.* **1993**, *291*, 529.
16. Ortwerth, B. J.; James, H.; Simpson, G.; Linetsky, M. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 161.
17. Chellan, P.; Nagaraj, R. H. *J. Biol. Chem.* **2001**, *276*, 3895.
18. Yim, H.-S.; Kang, S.-O.; Hah, Y.-C.; Chock, P. B.; Yim, M. B. *J. Biol. Chem.* **1995**, *270*, 28228.
19. Elgawish, A.; Glomb, M.; Friedlander, M.; Monnier, V. M. *J. Biol. Chem.* **1996**, *271*, 12964.
20. Akagawa, M., Sasaki, T. and Suyama, K. *Eur. J. Biochem.* In press.
21. Hayase, F.; Shibuya, T.; Sato, J.; Yamamoto, M. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1820.
22. Ahmed, M. U.; Thorpe, S. R.; Baynes, J. W. *J. Biol. Chem.* **1996**, *261*, 4889.
23. Akagawa, M.; Suyama, K. *Free Radic. Res.* **2002**, *36*, 13.
25. Schönberg, A.; Moubacher, R. *Chem. Rev.* **1952**, *50*, 261.
25. Hofmann, T.; Münch, P.; Schieberle, P. *J. Agric. Food. Chem.* **2000**, *48*, 434.
26. Shah, M. A.; Bergethon, P. R.; Boak, A. M.; Gallop, P. M.; Kagan, H. M. *Biochim. Biophys. Acta* **1992**, *1159*, 311.
27. Akagawa, M.; Suyama, K. *Biochem. Biophys. Res. Commun.* **2001**, *281*, 193.
28. Kato, H.; van Chuyen, N.; Utsunomiya, N.; Okitani, A. *J. Nutr. Sci. Vitaminol.* **1986**, *32*, 55.
29. Wells-Knecht, K. J.; Zyzak, D. V.; Litchfield, J. E.; Thorpe, S. R.; Baynes, J. W. *Biochemistry* **1995**, *34*, 3702.
30. Danshvar, B.; Frandsen, H.; Autrup, H.; Dragsted, L. O. *Biomarkers* **1997**, *2*, 117.
31. Requena, J. R.; Chao, C.-C.; Levine, R. L.; Stadtman, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 69.