Effects of 3-Deoxyglucosone on the Maillard Reaction

Naoya Igaki, Makoto Sakai, Hulmihiro Hata, Munetada Otomori, Shigeaki Baba, and Hiromichi Kato

We investigated the effect of exogenously applied 3-deoxyglucosone, a major carbonyl intermediate, on the Maillard reaction. The fluorescence intensity of the product of the reaction of bovine serum albumin with 3-deoxyglucosone was higher than that with an equivalent amount of glucose. Similarly, the rate of polymerization of lysozyme in the presence of 3-deoxyglucosone was also higher than with glucose, and collagen incubated with 3-deoxyglucosone was less digestible than collagen incubated with glucose. By contrast, aminoguanidine inhibited an increase in fluorescence of the Maillard compounds and the polymerization of protein, both of which were stimulated by 3-deoxyglucosone. These results suggest that 3-deoxyglucosone accelerates the advanced stage of the Maillard reaction and that aminoguanidine acts on 3-deoxyglucosone to inhibit its action in the advanced stage of the Maillard reaction.

Additional Keyphrases: diabetes mellitus • aminoguanidine • albumin • lysozyme • collagen

Glycation, a reaction in which reducing sugars are bound to proteins nonenzymatically and nonspecifically, is also called the Maillard reaction. Up to the formation of Amadori compounds, this process is considered as the early stage of the Maillard reaction, and the subsequent reactions, from the Amadori compounds onwards, are considered the advanced stage.

The Maillard reaction has attracted attention because of its possible involvement in the progression of diabetic complications and aging, in that the products of the advanced stage of the reaction accumulate in long-lived proteins, e.g., in collagen (1), nerve myelin (2), and lens crystalline (3).

In the advanced stage of the Maillard reaction, the proteins involved become fluorescent and alter in various ways, such as browning, increased cross-linking, loss of solubility, and reduction in digestibility. Pongor et al. (4) suggested some characteristics of advanced glycosylation end products of the advanced stage of the Maillard reaction, in particular the presence of 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole as one of the specific fluorescent products of the advanced reaction. However, Njoroge et al. (5) proposed that this compound was generated from the reaction between the furose produced by the acid hydrolysis of Amadori compounds and ammonia, and argued against its presence in living organisms. The identification of the advanced-stage Maillard products remains difficult, and little detailed information about them is available. Recently, 3-deoxyglucosone (3DG) has been suggested as a major carbonyl intermediate of Amadori compounds (6), and its possible role in the Maillard reaction has received some attention. In the present study, we investigated the effects of 3DG on the Maillard reaction.

Materials and Methods

Fluorescence Intensity

We used bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) as a model protein, incubating BSA at 1 g/L with 5 mmol/L glucose or 5 mmol/L 3DG solutions (7) in 500 mmol/L sodium phosphate buffer, pH 7.45, at 37 °C for 3, 7, 10, and 14 days. We determined the fluorescence intensity of the mixtures after each incubation interval.

After 14 days at the above-mentioned conditions, glycated BSA, formed by incubation with the glucose, was dialyzed against distilled water for 24 h to remove excess glucose. After removing the glucose, we incubated glycated BSA with 3DG (0.5, 1, and 10 mmol/L) for 14 days. We then determined the fluorescence intensity of these solutions.

A mixture of various concentrations of aminoguanidine (hemisulfate; Sigma) and 3DG in the above-mentioned buffer was preincubated for 24 h, then incubated with BSA, 1 g/L, for 14 days. The fluorescence intensity of the mixture was then determined.

The fluorescence intensity generated from the advanced-stage Maillard products was determined with a fluorescence spectrophotometer (Model 650-60; Hitachi Ltd., Tokyo, Japan) with an excitation wavelength of 370 nm and an emission wavelength of 440 nm (8). Fluorescence intensity is expressed in arbitrary units per milligram of protein. Protein was assayed according to the method of Bradford (9).

Rate of Polymerization

Lysozyme (6× crystallized, from egg white; Seikagaku Kogyo Co., Tokyo, Japan) was used as the model protein. We incubated lysozyme, 10 g/L, with 5 mmol/L solutions of glucose or 3DG for 28 days under the above-mentioned buffer conditions. The sample was then dialyzed against distilled water to remove excess glucose and 3DG, and then evaporated to dryness. We reconstituted the sample in 1 mL of sample buffer (per liter: 10 mmol of Tris HCl, 1 mmol of EDTA, 10 g of SDS, and 50 mL of β-mercaptoethanol, pH 8.0), boiled this for 10 min at 100 °C, and subjected the sample to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was conducted on a gradient gel (PAA 4/30; Pharmacia Co., Uppsala, Sweden) according to the method of Weber and Osborn (10). An electrophoresis calibration kit for determination of mobilities of low-molecular-mass proteins (Pharmacia Co.) was used as the source of internal standards. Gels were stained with Coomassie Brilliant Blue R-250 (Nakarai Tesque Co., Kyoto, Japan) and destained with ethanol/acetate (95/5 by vol).

The Second Department of Internal Medicine, Kobe University School of Medicine, 5-1, 7-chome, Kusunoki-cho, Chuo-ku, Kobe, 650, Japan.

1 Faculty of Agriculture, The University of Tokyo, Tokyo, Japan.

Received October 23, 1988; accepted January 19, 1990.

2 Nonstandard abbreviations: 3DG, 3-deoxyglucosone; BSA, bovine serum albumin; and SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.
Digestibility

For this study we used type I collagen (from bovine achilles tendon; Sigma) as the model protein, incubating a 20 g/L solution of collagen with 5 mmol/L solutions of glucose or 3DG under the above-mentioned buffer conditions. We then digested these samples with 200 U of type VII collagenase (EC 3.4.24.3; Sigma) for 48 h and divided them into soluble and insoluble fractions by centrifugation at 10 000 × g for 10 min. The amount of hydroxyproline in each fraction was determined by the method of LaBella and Paul (11), and digestibility was calculated as the ratio of hydroxyproline in the soluble fraction to the total hydroxyproline content.

Statistical Analysis

We used Student’s t-test for statistical analyses.

Results

When glucose or 3DG was incubated with BSA, the fluorescence intensity increased significantly in a time-dependent manner. After incubation for 14 days, the fluorescence intensity was 9.5 ± 0.680 units and 2.1 ± 0.140 units for 3DG-containing and glucose-containing reaction mixtures, respectively (P < 0.01). The fluorescence intensity was much greater when 3DG was present than when glucose was present (Table 1).

When aminoguanidine was preincubated with 3DG, the fluorescence intensity generated from BSA and 3DG was inhibited by aminoguanidine (original concentrations, 1 and 5 mmol/L) in a dose-dependent manner (Table 2).

After removal of excess glucose, glycated BSA was incubated with 3DG, 0, 5, and 10 mmol/L. The fluorescence intensity of glycated BSA was developed to the same extent regardless of the dose of added 3DG (Table 3).

The rate of polymerization of lysozyme protein induced by glucose or 3DG was analyzed by SDS-PAGE (Figure 1). The rate of 3DG-induced polymerization was greater than that induced by an equivalent amount of glucose. Moreover, the rate of 3DG-induced polymerization was diminished by preincubation of 3DG with aminoguanidine.

Examination of changes in the digestibility of collagen protein induced by 3DG (Table 4) showed respective values of 75 (SD 3.0)% and 96 (SD 0.42)% of the original digestibility for the 3DG-containing and glucose-containing reactions (P < 0.01). Aminoguanidine preincubated with 3DG resulted in a digestibility of 99 (SD 1.0)% of the original value (P < 0.01).

Discussion

Since the clinical application of hemoglobin A1c (12), the Maillard reaction has attracted attention not only in the field of food chemistry, but also in studies of the living organisms. This reaction has been studied with respect to diabetes, in particular with reference to associated complications (13).

The Maillard reaction is actually a complicated series of reactions, and the pathway from the advanced stage, beyond the formation of Amadori compounds, to the formation of the final product, a brown polymerized compound known as melanoidin, is so complicated it has not yet been unraveled precisely. Recently, Kato et al. (6) demonstrated that 3DG is a major compound among the various carbonyl compounds generated from Amadori compounds and plays an important role as an active intermediate in the Maillard reaction. The present study demonstrates that 3DG increases the fluorescence intensity, accelerates the polymerization, and induces less solubility of a model protein more markedly than an equivalent amount of glucose. These protein alterations are characteristic of advanced glycosylation end products. In addition, the fluorescence intensity of glycated BSA after removal of excess glucose increased to the same extent, regardless of the addition of exogenous 3DG. This result suggests that 3DG may be involved in the formation of Amadori compounds, or that the effect of exogenously added 3DG is offset by endogenous 3DG generated from Amadori compounds. Hayase et al. (14) indicated that 5-hydroxy-methyl-1-alkylpyrrole-2-carbaldehyde (also called pyrralline), generated from 3DG and free lysine, ...
Table 4. Digestibility of Collagen by Collagenase after Incubation with Glucose or 3DG

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Digestibility, %, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen alone</td>
<td>97 ± 0.90</td>
</tr>
<tr>
<td>+ Glucose, 5 mmol/L</td>
<td>98 ± 0.42</td>
</tr>
<tr>
<td>+ 3DG, 5 mmol/L</td>
<td>75 ± 3.30*</td>
</tr>
<tr>
<td>+ Glucose, 5 mmol/L + AG, 50 mmol/L</td>
<td>98 ± 0.68</td>
</tr>
<tr>
<td>+ 3DG, 5 mmol/L + AG, 50 mmol/L</td>
<td>99 ± 1.00</td>
</tr>
</tbody>
</table>

n = 3. AG, aminoguanidine. *Significantly different (P <0.01) from results for collagen alone and for collagen + 3DG + AG.

is one of the advanced-stage products of the Maillard reaction. They also suggested that there were no differences in the kinetics of the reaction between 3DG and glycated lysine and that the formation of pyralline from 3DG was the rate-limiting step in the reaction of BSA with glycated lysine.

Recently, Suarez et al. (15), McPherson et al. (16), and Oimomi et al. (17) demonstrated that fructose, generated from the polyol pathway, can play a potentially pathogenic role in tissue damage via the Maillard reaction: namely, fructose accelerated the advanced stage of the Maillard reaction more rapidly than glucose. The precise mechanism of this reaction is still unclear. However, Shin et al. (18) demonstrated that the rate of formation of 3DG in a protein–fructose system was 1.3–2.0 times that in a protein–glucose system and that 3DG was responsible for this difference. Linkage between the Maillard reaction and polyol pathway is important, and 3DG is a key compound in this regard.

Investigating how to prevent diabetic complications by suppressing formation of advanced-stage Maillard products, Brownlee et al. (19) focused on aminoguanidine and found that it inhibited the advanced stage of the Maillard reaction with collagen in vitro. They also found that aminoguanidine inhibited the development of fluorescence and cross-linking of proteins from arterial wall tissue in rats in vivo. They considered that aminoguanidine bound to Amadori compounds, leading to the formation of substituted (unreactive) Amadori compounds and hence the stabilization of Amadori compounds, thereby inhibiting subsequent continuation of the Maillard reaction. However, in the present study we have shown that aminoguanidine inhibits the action of 3DG, an intermediate in the Maillard reaction that acts as a strong cross-linker. This result suggests the diversity of the mechanisms by which aminoguanidine inhibits the advanced stage of the Maillard reaction. If aminoguanidine can be applied in living organisms, it might be useful in preventing diabetic complications.

Living organisms have various mechanisms to protect against the Maillard reaction. Vlassara et al. (20) found specific receptors for advanced glycosylation end products on macrophages. Oimomi et al. (21) extracted α-ketoaldehyde dehydrogenase from human liver and showed that 3DG was a substrate for this enzyme, thus demonstrating the significance of 3DG and an enzymatic regulatory system in the Maillard reaction. The physiological concentration, metabolism, and other characteristics of 3DG in living organisms clearly need to be elucidated.

In conclusion, our results suggest that 3DG is an activator of the Maillard reaction and that aminoguanidine inhibits the effects of 3DG, thereby inhibiting the advanced stage of the Maillard reaction.

References
14. Hayase F, Nagaraj RH, Miyata S, Njoroje FG, Monnier VM. Aging of proteins: immunological detection of a glucose-derived...