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EFFECT OF AMINOGUANIDINE ON THE GLYCATION

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INDEXING WORDS

aminoguanidine; glycation; 3-deoxyglucosone; Maillard reaction; diabetes mellitus

SYNOPSIS

3-Deoxyglucosone, a carbonyl intermediate compound in the Maillard reaction, acts on bovine serum albumin to increase its fluorescence.

Aminoguanidine inhibited the increase of fluorescence intensity formed by bovine serum albumin and 3-deoxyglucosone when 3-deoxyglucosone had been preincubated with aminoguanidine.

These results suggested that aminoguanidine inhibits the action of 3-deoxyglucosone in the Maillard reaction.

INTRODUCTION

Glycation in which sugars are bound non-enzymatically to proteins is also called the Maillard reaction.[5] The reaction up to the formation of Amadori compounds is considered to be the early stage of the Maillard reaction and the later reaction of the advanced stage.[6] Advanced stage products accumulate in long-lived proteins, such as collagen, myellin and lens,[9] and have attracted

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attention in relation to the progression of diabetic complications and aging.[3] These advanced stage Maillard products have fluorescence,[7] result in browning, and undergo a complicated series of reactions, such as polymerization and insolubilization, through cross-linking.[8] They are said to be transformed into melanoidin in vitro.[6]

In the present study, we investigated the effects of 3-deoxyglucosone (3-DG),[4] a major carbonyl intermediate in the Maillard reaction which is formed as a result of decomposition of Amadori rearrangement compounds, on the formation of Maillard products and on aminoguanidine, which has received attention because of its inhibitory effect on the formation of advanced Maillard products.

MATERIALS AND METHODS

Defatted bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, USA) was used as a model protein, and 0.015 mM BSA was incubated with 10 mM glucose (Wako Pure Chemical Industries Ltd., Tokyo) or 10 mM 3-DG (Kato et al.[4]) in a 500 mM sodium phosphate buffer, pH 7.45, at 37C for 14 days, and fluorescence was determined. Different concentrations of aminoguanidine (hemisulfate; Sigma Chemical Co., St. Louis, USA) were added to 3-DG and the mixture was preincubated under the above-mentioned buffer conditions for 24 hours, then incubated with 0.015 mM BSA for 14 days. Then, fluorophotometry was performed. For fluorophotometry, a fluorescence spectrophotometer (Hitachi 650-60; Hitachi Co., Tokyo) was used at an excitation wavelength of 370 nm and an emission wavelength of 440 nm.[7] The fluorescence intensity was expressed in arbitrary units/mg protein. Protein was determined by the method of Bradford.[1]

Student's t test was used for statistical analysis.

RESULTS

When glucose or 3-DG was added to BSA, the fluorescence intensity increased significantly. The fluorescence intensity was much higher when 3-DG was added than when glucose was added (Table 1).

Table 1 The fluorescence intensity after the incubation of bovine serum alubumin with glucose or 3-deoxyglucosone in 500 mM sodium phosphate buffer with 3 mM sodium azide, pH 7.45, at 37C for 14 days.

Incubation mixture	Fluorescence intensity (arbitrary units/mg protein)	
BSA	0.96±0.01	
BSA+10mM Glucose	2.10±0.14	P<0.01
BSA+10mM 3-DG	9.50±0.68	P<0.001

When 3-DG had been preincubated with aminoguanidine, the fluorescence intensity formed by BSA and 3-DG was inhibited by 1 and 5 mM aminoguanidine in a dose-dependent manner (Fig. 1).

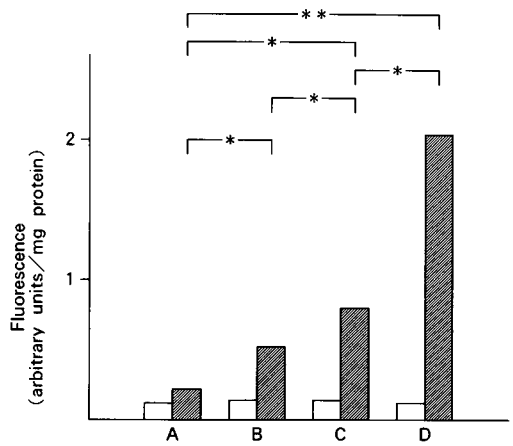

Fig. 1 The fluorescence intensity after the incubation of bovine serum albumin with the preincubated mixture of 3-deoxyglucosone and aminoguanidine in 500 mM sodium phosphate buffer, with 3 mM sodium azide, pH 7.45, at 37C for 14 days.
Hached column indicate the fluorescence intensity after the incubation.
Open column indicate the fluorescence intensity before the incubation of each group.
Preincubation mixture

	3-deoxyglucosone	Aminoguanidine
A:	non-addition	non-addition
B:	1 mM	5 mM
C:	1 mM	1 mM
D:	1 mM	non-addition

Each data represents the mean of 3 experiments.

DISCUSSION

Since the clinical applications of hemoglobin A_{1c} (HbA_{1c})[10,12] became apparent, attention has been paid to the Maillard reaction, not only in the field of food chemistry, but also in the living body.[6,11] This reaction has been studied with respect to its relation with diabetes, particularly diabetic complications.[3] On the other hand, the Maillard reaction goes through a complicated series of reactions and the pathway from the advanced stage following Amadori rearrangement to the formation of the final product, a brown polymerized compound melanoidin, is so complicated that much about it is not understood. Recently, it has been demonstrated that, among various carbonyl compounds formed from Amadori rearrangement compounds, 3-DG plays an important role as an active intermediate.[4] In fact, the present study revealed that 3-DG increased the intensity of fluorescence more markedly than glucose in equal amounts, indicating the importance of 3-DG in the formation of advanced products of the Maillard reaction.

For the purpose of preventing diabetic complications by inhibiting the formation of advanced Maillard products, Cerami et al.[2] focused on aminoguanidine and found that it inhibited the advanced stage of the Maillard reaction in collagen in vitro. They also demonstrated in an in vitro experiment in rats that aminoguanidine inhibited the fluorescence intensity and cross-linking arterial wall tissue protein. Aminoguanidine is considered to be bound to Amadori compounds, leading to the formation of substituted Amadori compounds (unreactive) and the stabilization of Amadori compounds, thereby inhibiting the subsequent Maillard reaction. However, the present study showed that aminoguanidine has an inhibitory effect on 3-DG, an intermediate Amadori compound in the Maillard reaction that acts as a strong cross-linker. This result suggests the diversity of mechanisms of action by which aminoguanidine inhibits the advanced stage of the Maillard reaction. If aminoguandine can be applied to the living body, it should be of great significance in the prevention of diabetic complications.

In conclusion, it was revealed that 3-DG is an activator of the advanced Maillard reaction and that aminoguanidine inhibits the effect of 3-DG, thereby inhibiting the advanced Maillard reaction.

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