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OIMOMI, MUNETADA

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THE EFFECT OF FRUCTOSE ON COLLAGEN GLYCATION

MUNETADA OIMOMI, MAKOTO SAKAI, TAKESHI OHARA,
NAOYA IGAKI, TSUNEO NAKAMICHI, SHIGEKI NISHIMOTO,
FUMIHIKO HATA, AND SHIGEAKI BABA

The Second Department of Internal Medicine
Kobe University School of Medicine

INDEXING WORDS

fructose; glycation; Maillard reaction; diabetes mellitus; polyol pathway

SYNOPSIS

The effect of fructose on the formation of advanced Maillard reaction products which have fluorescence and cross-links was investigated. Type I collagen was added to various concentrations of glucose and fructose which were then incubated at 37C for 4 weeks.

Both the level of furosine and the fluorescence intensity increased in direct proportion to glucose and fructose levels and to the duration of incubation. Incubation with fructose produced less furosine but more intense fluorescence than incubation with glucose.

These results suggest that fructose in the polyol pathway plays an important role in the formation of advanced Maillard products.

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Authors' names in Japanese : 老穀宗忠, 坂井 誠, 大原 毅
井垣直哉, 中道恒雄, 西本茂樹
秦 文彦, 馬場茂明

INTRODUCTION

The nonenzymatic binding of sugars to proteins is called glycation, and the process through which such binding occurs is known as the Maillard reaction.⁷⁾ The Maillard reaction has been studied previously mainly in the field of food chemistry.⁸⁾

Triggered by the clinical application of glycated hemoglobin,^{1,14)} glycation has received attention not only because it provides an indicator of the level of glucose in the blood of diabetic patients but also because it may be related to the development of diabetic complications, aging, and arteriosclerosis.^{4,6)} The Maillard reaction progresses slowly to produce advanced-stage products, not only in vitro, such as in food chemistry, but also in the living body, with repeated dehydrations and rearrangements, resulting in browning products which have fluorescence and cross-links.¹¹⁾

The advanced stage Maillard products continue to accumulate in long-lived proteins such as collagen over a long period of time.¹²⁾ Therefore, these late stage products may be more closely related to the degradation of tissue proteins than the early-stage products of the Maillard reaction.

In the present study, we investigated the effects of glucose and fructose on the formation of Maillard reaction products, i.e., furosine as an early-stage product¹³⁾ and fluorescence as an indicator of the presence of advanced stage Maillard products.¹⁰⁾ The early-stage products of the Maillard reaction increase with larger amounts of protein and glucose and this step of the reaction is especially accelerated by the presence of sugars besides glucose, such as glucose-6-phosphate.³⁾ In the living body, we ingest sugars like glucose, fructose and sucrose. Thus, in the present study, we considered not only glucose but also fructose. We investigated the effect of fructose on the formation of advanced stage Maillard products.

MATERIALS AND METHODS

To 10 mg/ml of Type I collagen derived from bovine achilles' tendon (Sigma Chemical Co., St. Louis, USA) we added D-glucose whose concentration was 100 or 200 mM, or D-fructose whose concen-

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tration was 50, 100 or 200 mM, respectively. Each mixture was incubated in 0.02 M HEPES buffer at pH 7.5 with same physiological conditions and distilled water was stirred in twice. After the centrifugation of the mixture the supernatant was collected and dialysed in distilled water for 48 hours. Then, glycated collagen was purified.

Half of the glycated collagen, about 10 mg, was acid-hydrolysed with 6 N HCl for 30 hours at 95C and the hydrolysate was evaporated. The dried sample was subsequently dissolved in about 200 μ l of distilled water. The solution which resulted was passed through a membrane filter (pore size 0.2 μ ; Millipore Corp., Bedford, MA, USA).

Furosine was measured by high-performance liquid chromatography (HPLC) (Waters Ltd., Co., Tokyo, Japan) on an ODS-120A column (Toyo Soda Co., Ltd., Tokyo, Japan), 4.6 mm x 25 cm. As a solvent 7 mmol/l H_3PO_4 was used. The chromatographic conditions were as follows: flow rate, 1 ml/min; detector sensitivity, 0.04 absorbance unit of full scale; UV detector wavelength, 280 nm and 254 nm. The ratio of the furosine peak area at 280 nm to that at 254 nm was 3.9:1.¹³⁾ The furosine content was expressed as the ratio of the furosine peak area to the tyrosine peak area.

The other half of the glycated collagen was resuspended in 1 ml of 0.02 M HEPES buffer at pH 7.5 containing 100 units of collagenase (Sigma Chemical Co., St. Louis, USA) and was digested at 37C for 48 hours. Five μ l of chloroform and 5 μ l of toluene were each added to prevent bacterial growth. After the centrifugation 10,000 x g for 20 min, 5 ml of the supernatant was collected and the samples were diluted in distilled water which was 5 times the amount used for the determination of the fluorescence intensity.

The fluorescence intensity of each sample was measured at the excitation wavelength of 370 nm and the emission wavelength of 440 nm. Fluorescence levels were expressed in arbitrary units per milligram of collagen. Collagen content estimated by measuring the amount of hydroxyproline.

Statistical analysis was performed with Student's t test.

RESULTS

Both the level of furosine and the fluorescence intensity

increased with the concentration of glucose or fructose and with incubation time. The level of furosine was significantly lower after incubation with fructose than after incubation with glucose (Fig. 1). However, the fluorescence intensity was significantly higher after incubation with fructose than after incubation with glucose (Fig. 2).

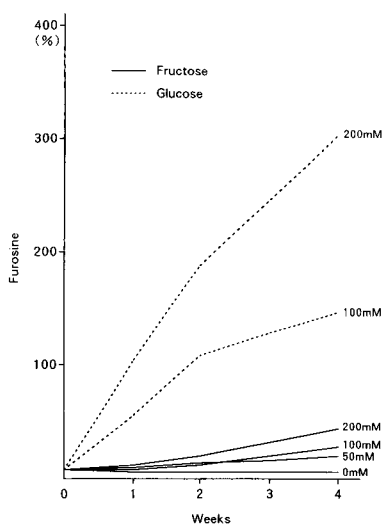


Fig. 1
Furosine levels after incubation of collagen with various concentrations of fructose (—) or glucose (-----) at 37C for 4 weeks. The data represent the mean of 3 experiments.

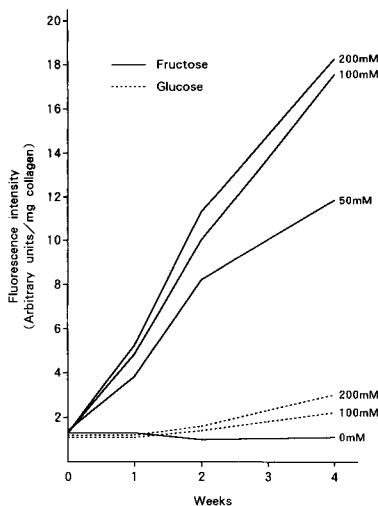


Fig. 2
Fluorescence intensity after incubation of collagen with various concentrations of fructose (—) or glucose (-----) at 37C for 4 weeks. The data represent the mean of 3 experiments.

DISCUSSION

In the present study we used Type I collagen as an example of interstitial collagen; it is the most abundant collagen in the human body. However, because glycation is non-enzymatic and non-specific reaction, one might assume that it takes place at similar rates among other types of collagen as well.

Our furosine measurements revealed that fructose incubation produced less Amadori product but more advanced Maillard product than did glucose incubation. We think glucose bound at C₁ and

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fructose at C₂.⁹⁾ Fructose then, presumably, bound to protein (fructosylation), forming Heyns rearrangement products,⁵⁾ which are intermediates of and produce advanced-stage products of the Maillard reaction.

In the polyol pathway,²⁾ a recent focus in diabetic complications, more glucose leads to more sorbitol and to the formation and accumulation of fructose in tissues. Therefore, if fructose leads to advanced-stage Maillard products as we believe it does, the polyol pathway too may contribute to the amount of advanced-stage Maillard products in the body.

Our results suggested that advanced Maillard products produced from fructose accumulate in tissues such as collagen and cause diabetic complications via the polyol pathway.

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