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GLYCOSYLATED COLLAGEN

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SUMMARY

Collagen was separated from segments of thoracic aorta excised from normal and streptozotocin-induced diabetic rats. The extent of collagen glycosylation was determined using a colorimetric chemical procedure specific for the detection of ketoamine-linked hexoses in proteins. Diabetic rats exhibited a significant increase in glycosylated collagen as compared to normal animals. Glycosylated collagen may contribute to the development of diabetic vascular complications.

INTRODUCTION

Collagen, the main fibrous protein of connective tissue, is the most abundant protein in the human body where it occurs primarily as extracellular, insoluble fibers. These fibers account for the major part of the organic mass of skin, bone, tendon, and blood vessels (1). The unique structural and functional properties of collagen depend on intra and intermolecular cross-linking of collagen fibrils (2). The formation of collagen cross-links is attributable primarily to the presence of lysine and hydroxylysine residues which may be subjected to increased glycosylation in the diabetic state. In order to gain insight into the relationship between hyperglycemia and vascular complications of diabetes, we initiated studies on the glycosylation of arterial collagen.

The demonstration in diabetics of increased amounts of the glycosylated form of hemoglobin A, hemoglobin A_{1c}, was the first example of posttranslational protein modification correlated with elevated blood glucose concentrations (3). It would seem reasonable to suppose that this type of nonenzymatic glycosylation

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also occurs with proteins of other tissues. Evidence has recently been published to link the development of corneal opalescence in diabetes with glycosylation of lens crystallins (4). Moreover, the nonenzymatic glycosylation of human serum proteins has also been reported (5).

Mechanistically, the nonenzymatic interaction of glucose and protein occurs by way of a ketoamine linkage, involving free amino groups at the N-terminus or ε-amino groups of lysine residues (6). The degree of protein glycosylation can be readily estimated by a colorimetric procedure which appears to be specific for hexoses bound to proteins by the ketoamine linkage (7). In this procedure, 5-hydroxymethylfurfural (HMF) generated from the carbohydrate moieties on heating under acidic conditions is quantitated colorimetrically with 2-thiobarbituric acid (TBA).

MATERIALS AND METHODS

Young adult female rats (Sprague-Dawley-derived strain), weighing 120-130 g, were rendered diabetic by a single tail vein injection of streptozotocin, 60 mg/kg, (8). The diabetic state was monitored by measuring urine glucose with Tes-Tape (Eli Lilly Co., Indianapolis, Ind.). Animals that excreted at least 0.5% glucose in the urine were judged to be diabetic. Six weeks after streptozotocin administration, the animals were sacrificed, 4 cm segments of thoracic aorta were excised, freed from extraneous tissues, and flushed with isotonic saline. Each aortal segment was defatted by successive immersions in acetone and ether for 18 h, dried for 24 h at 100°C, and weighed.

Collagen was separated by autoclaving each aortal specimen in 4 ml distilled water for 18 h at 15 lb/in^2 and decanting the extract (9). The amount of collagen in each extract was determined from its hydroxyproline content following the autoclaving of an aliquot of the extract in 6 N HCl for 18 h at 15 lb/in^2 (10). To obtain the quantity of collagen, the hydroxyproline content was multiplied by a factor of 7.46 (11).

The TBA reaction for determining the presence of HMF was performed by mixing 1 ml of collagen extract with 0.5 ml 0.3 N oxalic acid and heating for 1 h in a boiling water bath. After cooling to room temperature, 0.5 ml 40% trichloroacetic acid was added and the resulting precipitate removed by filtration. After the addition of 0.5 ml of 0.05 M TBA, the solution was incubated at 40°C for 30 min and the absorbance was measured at 443 nm. The concentrations of HMF in test solutions were calculated from standard curves.

RESULTS AND DISCUSSION

Table 1 compares the liberation of HMF from arterial collagen obtained from normal and streptozotocin-induced diabetic rats. The amount of HMF released represents ketoamine-linked hexoses in collagen, and is therefore a

Table 1. Liberation of 5-hydroxymethylfurfural (HMF) from collagen extracted from thoracic aortas of normal and streptozotocin-induced diabetic rats. Values are the mean of 4 animals ± SD.

Source	n moles HMF / mg dry defatted tissue	n moles HMF / mg collagen
Normal	3.07 ± 0.505	14.37 ± 2.46
Diabetic	5.25 ± 0.550*	25.16 ± 4.17†

* $p < 0.005$

† $p < 0.05$

measure of the extent of collagen N-glycosylation. Rats with acute diabetes of six weeks duration show a greater degree of arterial collagen glycosylation than normal animals. This finding is not unexpected since vascular collagen is constantly exposed to high blood glucose concentrations characteristic of the diabetic state. Reactions between glucose and ε-amino groups of lysine and hydroxylsine are likely to occur.

It is interesting to note that N^{ε}-glycosyllysine and N^{ε}-mannosyllysine have been reported to be liberated following acid hydrolysis of calf skin collagen (12). This strongly indicates that ketoamine linkages between glucose and ε-amino groups of lysine residues do normally occur in collagen. Acid hydrolysis of collagen would result in racemization of the second carbon atom of the carbohydrate moiety and cause the formation of both N^{ε}-glucosyllysine as well as the C-2 epimer, N^{ε}-mannoxyllysine.

The nonenzymatic glycosylation of ε-amino groups of lysine and hydroxylysine in collagen is important, for it implies that these glucose-linked amino acids are no longer available for cross-linking. In diabetes, the increased glycosylation of collagen fibrils may alter the function, structure and turnover of collagen. The possibility that increased glucose concentrations directly contribute to the development of diabetic vascular complications by glycosylating collagen deserves further scrutiny.

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