NONENZYMATIC GLYCOSYLATION OF PROTEINS AND PROTEASE ACTIVITIES IN GRANULATION TISSUES IN EXPERIMENTAL DIABETES

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Abstract—Serum proteins and hemoglobins show increased nonenzymatic glycosylation in diabetes mellitus. The measure of glycosylated proteins, particularly hemoglobin, is considered to be a preferred indicator in the control of diabetes. In a study of diabetes and inflammation, we assessed the extent of nonenzymatic glycosylation of proteins of granulation tissue from diabetic rats. Five, seven, and ten days after carrageenan injection, the granuloma proteins were extracted. Nonenzymatic glycosylation was measured in soluble and insoluble granuloma proteins by thiobarbituric acid assay. Protease activities and free amino groups were assayed in soluble extracts. Nonenzymatic glycosylation in soluble proteins of both groups reached a maximum on the seventh day. However, nonenzymatic glycosylation in soluble proteins of the diabetic granulomas was significantly greater than the controls on days five and seven. During the days after granuloma induction, nonenzymatic glycosylation in the insoluble granuloma tissue proteins gradually decreased without any significant differences between controls and diabetics. Significant decreases in the free amino groups in soluble proteins of the diabetic tissues were noted. Greater activities of cathepsins B and D were noted in diabetic tissues over controls. These observations suggest that, in addition to increased proteolysis, increased nonenzymatic glycosylation of tissue proteins could be associated with the impaired process of wound healing in diabetics.

INTRODUCTION

Nonenzymatic glycosylation of proteins occurs by direct chemical reaction between reducing sugars and primary amino groups in proteins. The reaction proceeds through Amadori rearrangement to yield a stable ketamine derivative of the protein (1). Nonenzymatic glycosylation is a common posttranslational
modification of body proteins including crystallins (2), insulin (3), basic myelin proteins (4), membrane proteins (5, 6), collagen (7, 8), albumin (9–11), immunoglobulins (12, 13), and hemoglobin (14, 15).

Chronic hyperglycemia in diabetes enhances this posttranslational protein modification. Although the reaction has not been shown to have a direct pathologic sequelae, a measure of this process has indeed provided a better marker for glycemic control than an estimation of fasting blood sugar levels.

Nonenzymatic glycosylation of proteins has been shown to alter the nature and function of proteins. In hemoglobin the nonenzymatic glycosylation reaction alters electrophoretic mobility and, in a minor way, oxygen-carrying capacity (16). The glycosylation of apolipoproteins has been shown to decrease clearance of low-density lipoproteins in man (17). On the other hand, in animal models nonenzymatic glycosylation increases the catabolism of high-density lipoproteins (18). It also has been shown that nonenzymatic glycosylation of fibrinogen reduces its survival time in diabetes (19). Thus, nonenzymatic glycosylation of cellular proteins has been suggested as one of the possible causations in the pathogenesis of several diabetic complications (20).

Among the many complications, an impairment in wound healing is known to occur during uncontrolled diabetes mellitus (21). In order to assess whether extracellular matrix proteins are nonenzymatically glycosylated during wound healing in diabetes, we studied the nonenzymatic glycosylation of soluble and insoluble granuloma tissue proteins using carrageenan granuloma as a model for inflammation. Ketamine-linked hexoses of the proteins are known to be converted to 5-hydroxymethylfurfuraldehyde (HMF) in the presence of weak acids such as acetic acid (22, 23). Using thiobarbituric acid reaction (22), nonenzymatic glycosylation in proteins was assessed in normal and diabetic rats during progression and regression of granulomas.

**MATERIALS AND METHODS**

Sprague-Dawley male rats, weighing 300–350 g, were studied. Diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg body wt). Following treatment, rats were allowed to drink 5% glucose solution overnight to prevent transient hypoglycemia (24). Forty-eight hours after injection of streptozotocin, the animals were fasted overnight. Blood glucose levels were determined by Dextrostix strips (Ames Division, Miles Laboratories). Animals that exhibited greater than 175 mg/dl blood sugar were used in this experiment. Three weeks after the animals were made diabetic, granulomas were induced with carrageenan. Age-matched normal rats were used as controls.

**Induction of Granulomas.** Granulomas were induced in both control and diabetic rats by injection, into the subcutaneous tissue of the backs, with a 20 ml (1.5% w/v) solution of carrageenan (Sigma Chemical Co., St. Louis, Missouri) in 0.9% NaCl. Carrageenan was dissolved in saline at 60–80°C and injected after cooling within a temperature range of 30–40°C.

Control and diabetic rats were anesthetized with ether at five, seven, and ten days after the induction of granuloma. Blood was drawn through the inferior vena cava, serum was separated,