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Nonenzymatic Galactosylation of Human Serum Albumin

IN VITRO PREPARATION*

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Incubation of purified human serum albumin with D-[1-14C]galactose (5 mM) or D-[1-14C]glucose (5 mM) in vitro for 7 days under physiological conditions resulted in the time-dependent accumulation of radioactivity into trichloroacetic acid-precipitable material. Comparative studies indicated that the rate of sugar incorporation into albumin increased with increasing pH and temperature of incubation and followed a first order dependence with regard to monosaccharide and albumin concentrations. The extent of nonenzymatic galactosylation of human albumin was approximately 300% greater than the extent of nonenzymatic glucosylation under equivalent experimental conditions. Prolonged dialysis of the modified albumins against a large excess of the unlabeled monosaccharides failed to alter the amount of protein-bound radiolabeled carbohydrate, suggesting that the linkage between sugar and albumin is covalent in nature. The post-translational modification of proteins by nonenzymatic galactosylation may be of physiological significance in individuals with reduced galactokinase or galactose-1-phosphate uridyl transferase activities.

The increasing amount of reports with regard to the nonenzymatic incorporation of glucose into proteins, including lens crystallins (1-4), insulin (5), basic myelin protein (6), erythrocyte membrane proteins (7, 8), collagen (9-11), albumin (12-15), immunoglobulins (13, 14), and hemoglobin (16-20), has encouraged speculation upon the significance of this type of post-translational protein modification. For example, quantities of hemoglobin A2c, a nonenzymatically glycosylated form of hemoglobin A, parallel blood glucose concentration (25-27). In addition, the nonenzymatic glycosylation of lens crystallins is implicated in the etiology of both diabetic and galactosemic cataracts (1, 2, 28, 29). It has been suggested that the clinical sequelae of diabetes may accrue from alterations in protein function occurring as a result of glucose-dependent chemical modification (3). At the present time, the relationship between elevated tissue galactose concentrations and observed pathological complications in galactosemic patients remains obscure. However, we suggest that the hypothesis offered for diabetes may also be applied regarding galactosemia.

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We present here a preliminary report of the nonenzymatic galactosylation of human serum albumin in vitro, and in addition, we demonstrate that human albumin incorporates galactose both faster and more extensively than glucose under various experimental conditions. A partial description has already appeared (30).

EXPERIMENTAL PROCEDURES

Materials—Human serum was obtained fresh from the University of Rhode Island Health Center (Kingston, RI). Human serum albumin was purified from fresh human serum by affinity chromatography on Affi-Gel blue (Bio-Rad) (31), followed by gel filtration on Bio-Gel P-150 (Bio-Rad) prior to recrystallization (32). D-Galactose and d-glucose were purchased from Sigma. D-[1-14C]Galactose (6.2 mCi/mmol) was obtained from New England Nuclear, D-[1-14C]Glucose (5.8 mCi/mmol) was purchased from International Chemical and Nuclear. Both chemical and radiochemical purity of the labeled sugars was assured at greater than 98% by the manufacturers; subsequent chromatographic and radiographic analysis in our laboratory confirmed the integrity of the tracers.

Incubations—All solutions and media were sterilized by autoclaving or by ultrafiltration prior to incubation at 37 °C in a shaking water bath. Sodium azide (0.02%) was added to prevent bacterial growth. Unless indicated otherwise, human albumin solutions (10 mg/ml) were prepared in Dulbecco’s phosphate-buffered saline, pH 7.4 (33), containing either galactose or glucose at a concentration of 5 mM. Trace amounts of the radiolabeled sugars were added to the mixtures to obtain desired specific activity.

Assays—Protein-bound radioactivity was measured by removing 0.04-ml aliquots of incubation mixtures to 0.05 ml of a bovine serum albumin solution (10 mg/ml) and precipitating with 1.0 ml of cold 10% trichloroacetic acid. Precipitates were dissolved in 0.5 ml of Protosol (New England Nuclear) and counted for radioactivity in Aquasol-2 (New England Nuclear). Protein was assessed by the method of Lowry et al. (34) with human serum albumin serving as the standard.

RESULTS

Fig. 1 compares the incorporation of radionuclide into acid-precipitable material upon reaction of purified human serum albumin with either D-[1-14C]galactose or D-[1-14C]glucose. Protein accumulation of radioactivity followed a rectangular hyperbola with increasing incubation for 7 days, at which time approximately 1.6 mol of galactose or 0.5 mol of glucose was incorporated per mol of albumin. Despite the equivalence of reaction conditions, both the initial velocity and the degree of association of radionuclide with acid-precipitable material were significantly greater when galactose was employed relative to when glucose was substituted in the incubations. It should be noted that chromatography of a hydrolysate of albumin, obtained at 7 days from the incubation with galactose, described in Fig. 1, resulted in the recovery of radiolabel both galactose and talose in a ratio of 3:1. The observation is not unexpected since the proposed reaction mechanism
involving an Amadori rearrangement to the ketoamine adduct would destroy the chiral center at C-2 of galactose. Consequently, both galactose and talose should be obtained upon protein hydrolysis. This experiment strongly suggests that galactose, and not an alleged radiolysis contaminant (35), is indeed the modifying agent of albumin, at least with concern to the research here described. In addition, the ratio of galactose to talose, 3:1, compares favorably with the ratio of glucose to mannose, 3:1, obtained upon hydrolysis of hemoglobin A_1C (21).
We decided next to compare the nonenzymatic galactosylation and the nonenzymatic glucosylation of human albumin in vitro with regard to both the extent and the rate of protein incorporation of monosaccharide as a function of various reaction conditions. Figs. 2 through 7 inclusive depict data concerning several of the kinetic and thermodynamic properties of albumin glycosylation in vitro. For example, the rate of incorporation of both galactose (Fig. 2) and glucose (Fig. 3) increased linearly with increasing concentration of the sugar; however, despite the equivalence of reaction conditions, nonenzymatic galactosylation occurred significantly faster than nonenzymatic glucosylation. Similarly, when protein glycosylation occurred in the presence of augmented concentrations of albumin, the linear increase in the rate of nonenzymatic galactosylation (Fig. 4) was again substantially greater than any comparative increase in the rate of glucose incorporation (Fig. 5) even though identical reaction conditions were employed. The experimental results described in Figs. 2 to 5 are not surprising, since the reaction mechanism would suggest that the initial rate of albumin glycosylation in vitro is first order with regard to both protein and sugar concentrations separately and second order overall.

Additionally, we attempted to make a comparison of the rates of nonenzymatic galactosylation and nonenzymatic glucosylation of human serum albumin in vitro under various conditions of pH and temperature. As shown in Figs. 6 and 7, protein incorporation of monosaccharide increased with both increasing pH and increasing temperature of incubation, re-
It is conceivable that the galactose-dependent chemical modification of proteins in *vitro* might alter both their rate and their path of metabolism, in addition to their proper functioning in the body. For example, the nonenzymatic galactosylation of albumin may affect its ability to transport metabolites such as bilirubin. Indeed, further investigation concerning this phenomenon may provide insight regarding the pathophysiology of certain disorders of carbohydrate metabolism.

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