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ROLE OF NONENZYMATIC GLYCOSYLATION IN EXPERIMENTAL CATARACT FORMATION

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SUMMARY

The relevance of nonenzymatic glycosylation of lens proteins to cataract formation was studied in rats on a normal and high galactose diet, treated with and without sorbinil, an aldose reductase inhibitor. All galactosemic rats not receiving sorbinil had cataracts; none receiving sorbinil had cataracts. Lens homogenate was treated with a 200 fold molar excess of [^{3}H]-borohydride and the extent of glycosylation was estimated from radioactivity incorporation and quantitation of hexitol-lysine adduct after extensive dialysis. We found no differences in the radioactivity uptake nor the amounts of hexitol-lysine in the lenses of galactosemic rats treated with and without sorbinil. Thus, nonenzymatic glycosylation was not responsible for the sugar-induced cataracts.

INTRODUCTION

Cataractous lenses in experimental sugar-induced diabetic or galactosemic animals have been shown to contain excessive amounts of sugar alcohol such as sorbitol or galactitol (1-3). Sorbitol and galactitol are formed by the action of aldose reductase (4,5) on glucose and galactose, respectively. Excessive accumulation of these

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sugar alcohols are known to exist in the lens epithelium and fiber cells, leading to tissue swelling and opacity by osmotic stress. Aldose reductase is considered as the key initiator of cataractogenesis in diabetes and galactosemia (2). In support of this, inhibitors of this enzyme were found to delay cataract formation in both galactosemic and diabetic rats (6-8).

In spite of abundant data from animal models demonstrating the potential role of aldose reductase in sugar-induced cataract formation, recent reports (9,10) have suggested an alternative explanation for the formation of sugar cataracts. They presented preliminary data indicating that nonenzymatic glycosylation, which is a well established phenomenon in hemoglobin (11), also occurred in lens crystallins and this structural alteration may predispose the lens crystallins toward cataract formation. Two previous reports (12,13) on human and galactosemic rat lenses found no significant correlation between the extent of glycosylation and cataract formation. However, the possible contribution of nonenzymatic glycosylation to the formation of sugar-induced cataracts remains to be answered. The primary aim of our experiments is to determine to what extent lens crystallins are modified by nonenzymatic glycosylation in vivo and to assess whether this phenomenon has any effect on the pathogenesis of cataract induced by galactose diet.

MATERIALS AND METHODS

Rats were made galactosemic by feeding them a 50% galactose diet. Drug-treated rats received sorbinil (14) (60 mg/kg/day) mixed with their food. Another control

group of rats received neither galactose nor sorbinil. Lenses of three experimental groups were extracted at different times as indicated in Table 1. Lenticular opacities of galactosemic and drug-treated rats were determined in each eye by slit lamp examination before they were sacrificed. Each pair of lenses was then homogenized in 5 ml of 0.065 M phosphate buffer (pH 6.8) with 0.2% SDS and 0.5% β-mercaptoethanol and centrifuged at 27,000g for 30 min. The supernatant was treated with 200 fold molar excess (based on 20,000 molecular weight) of [^{3}H]$NaBH_4$ (10.1 mci/mmole) in 0.1 N NaOH for 30 min. at 4°C. Each reaction mixture was lyophilized following extensive dialysis with several changes against two liters of distilled water until the radioactivity of the solution outside the dialysis bag fell to a negligible amount. The lyophilized lens proteins were then analysed for protein content with Lowry's method (15) and incorporation of radioactivity. The extent of glycosylation was estimated from a 6 N HCl protein hydrolysate by analyzing the hexitol-lysine adduct on a Durrum DC-6A cation exchange column.

RESULTS AND DISCUSSION

Three stages of cataracts in galactosemic rats were examined to determine the relationship between lens opacities and the degree of glycosylation of lens proteins. The vacuolar stage appeared within one week after the initiation of the galactose diet. By two weeks a dense nuclear opacity developed, and by four weeks the opacity involved the entire lens. In the sorbinil-treated galactosemic rats the lenses remained clear. Even after four weeks the lenses of galactose-fed rats were free of vacuoles and opacities.

Table 1 shows the specific radioactivity incorporation of three groups of lenses extracted at different times. The specific ^{3}H-radioactivity found in control rat lenses without galactose diet was about 1/3 - 1/2 those of galactosemic and inhibitor-treated lenses. There was a gradual increase of radioactivity in these latter two groups. No substantial difference in radioactivity incorporation

TABLE 1

SPECIFIC RADIOACTIVITY OF NaB^3H_4 INCORPORATION IN GALACTOSEMIC RATS TREATED WITH AND WITHOUT SORBINIL

Experiments	Days with Galactose Feeding	Specific Radioactivity (cpm/mg protein) 50% Galactose	50% Galactose + Sorbinil[a]	Control
I	7	16,200 ± 600[b]	18,600 ± 400	-
	14	24,800 ± 400	23,300 ± 700	12,400 ± 400
	21	25,100 ± 600	26,000 ± 800	-
	28	30,000 ± 900	31,200 ± 800	11,700 ± 300
II	7	17,800 ± 1000[c]	17,200 ± 800	-
	28	36,200 ± 2300	41,400 ± 2100	15,200 ± 500

a None receiving sorbinil treatment had cataract or vacuoles developed in the lenses.

b The radioactivity incorporation is expressed as counts per minute (cpm) per milligram protein content (Lowry's protein determination). Each value shown is the mean of duplicate analyses from paired lenses.

c Each value shown in Experiment II is the mean of analyses on three pairs of lenses using $[^3H]$ $NaBH_4$ with a specific radioactivity different than that in Experiment I.

could be found, which indicates that nonenzymatic glycosylation is not involved in triggering cataract formation. This conclusion is further strengthened by the finding that about equal amounts of $[^3H]$galactitol-lysine was detected in the 6 N HCl hydrolysates of crystallins of lenses from the sorbinil-treated and untreated galactosemic rats (Fig.1). The radioactivity incorporation and the galactitol-lysine detected should reflect the sites of lens crystallins glycosylated during 2-4 weeks of galactose

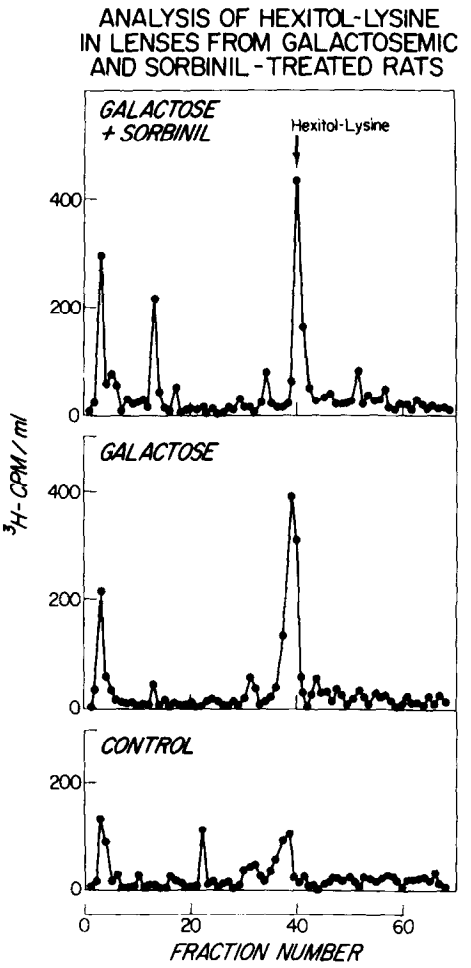

FIGURE 1. Separation of ^{3}H-labeled hydrolysates of rat lens proteins by high pressure cation exchange column (lenses from rats of 28-day feeding in Table 1). Upper panel, sorbinil-treated galactosemic rat lens protein hydrolysate (0.45 mg protein content and 13,900 cpm ^{3}H-radioactivity); middle panel, galactosemic rat lens protein hydrolysate (0.42 mg protein content and 12,600 cpm ^{3}H-radioactivity); lower panel, control rat lens protein hydrolysate (0.42 mg protein content and 4900 cpm ^{3}H-radioactivity). The hydrolysate was applied to the column (0.9 x 17 cm) in the starting equilibration buffer (0.2 M pyridine acetate, pH 3.2) and the elution was run for the first nine fractions followed by a 200 ml linear gradient of 0.2 M pyridine acetate, pH 3.2 to 1.5 M pyridine acetate, pH 5.0. Column pressure was maintained at 200-400 p.s.i. Fractions were collected at 2.8 ml per tube per 2.5 minutes. Total radioactivity recovery from the column was about 75% in each case. The arrow indicates the elution position of hexitol-lysine.

diet since [^{3}H]$NaBH_4$ is highly reactive with ketoamine linkages between reducing sugars and ε-amino groups of lysine (16). Sorbinil, a potent aldose reductase inhibitor, was shown to produce a 96-99% decrease in polyol accumulation in the lenses of galactosemic rats (17). That this was not a consequence of inhibited sugar transport was suggested by the observation that it had no effect on either fasting blood glucose concentration or glucose tolerance test (17). Recently, sorbinil has been shown to completely prevent cataracts in diabetic and galactosemic rats (18).

An initial study was conducted on lenses of streptozotocin-induced diabetic rats treated with and without sorbinil; we could find no difference in the ^{3}H-radioactivity incorporation but the amount of glucitol-lysine detected was much smaller than that of galactitol-lysine shown here. A likely explanation is that nonenzymatic glycosylation requires the reducing sugar with free aldehyde group in order to form a Schiff base with the ε-amino group of lysine. D-glucose has a much smaller proportion of free chain form than D-galactose (19), and is therefore less reactive with amino groups.

We have studied nonenzymatic glycosylation of bovine lenses of different ages. We found that nonenzymatic glycosylation is a general characteristic of the aging process of lens crystallins The amount of glucitol-lysine detected increased with the age of the animal.

In summary, studies on galactosemic and diabetic rat lenses clearly indicate that cataract development in these animal models is independent of nonenzymatic glycosylation of

lens crystallins in vivo. Lenses exposed to a high level of galactose in the presence of an aldose reductase inhibitor remain clear in spite of the same extent of glycosylation as cataractous galactosemic lenses. This finding excludes a significant role of nonenzymatic glycosylation in experimental sugar cataracts.

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