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Sorbitol Pathway in Lenses of Normal and Diabetic Rabbits

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The concentrations of sugars, D-glucose, D-sorbitol, D-fructose and *myo*-inositol, and the activities of enzymes, aldose reductase and sorbitol dehydrogenase, which are associated with the sorbitol pathway, in the lenses of normal and alloxan-diabetic rabbits were determined. It has been demonstrated that the formation of sorbitol and fructose is accelerated and the level of *myo*-inositol is decreaed in diabetic rabbit lens. The concentrations of glucose, sorbitol, fructose and *myo*-inositol in normal rabbit lens were about 0.23, 6.5, 0.3 and 6.0 μmol/g wet weight of lens, and those in diabetic rabbit lens were about 0.45, 29, 3 and 0.24 μmol/g wet weight of lens, respectively. It has been suggested that the levels of aldose reductase and sorbitol dehydrogenase activities in whole lens are unaffected by diabetic conditions. However, we found that the distribution pattern in the lens of aldose reductase was different under normal and diabetic conditions. In the diabetic condition, the level of the enzyme in the outer zone including the capsule, epithelium and cortex, increased, and that in the nucleus decreased.

Keywords———sorbitol pathway; aldose reductase; sorbitol dehydrogenase; diabetic rabbit; lens; cataract; diabetic cataract

The sorbitol pathway consists of two enzymes, aldose reductase [EC 1.1.1.21] and sorbitol dehydrogenase [EC 1.1.1.14], which catalyze the conversion of glucose to fructose by way of sorbitol. Aldose reductase in the lens plays a leading role in the etiology of diabetic cataract.[1,2] The presence of this enzyme in the diabetic lens, combined with a high glucose level, results in a high intracellular sorbitol level and a consequent uptake of water which leads to the opacification of lens, namely diabetic cataract. With respect to the accumulation of sorbitol in diabetic lens, Varma and Kinoshita[3] have reported that the acceleration of the sorbitol pathway, in other words a significantly higher aldose reductase level, was induced in the lens of rats in the diabetic condition. On the other hand, the elevation of aldose reductase level due to diabetic condition has not been observed for human lens.[4] Thus, some uncertainty regarding the contribution of the sorbitol pathway to the formation of diabetic cataract still exists. This uncertainty arises from inconsistent and limited data regarding the enzyme levels in animal lens.

The present report deals with studies of the effect of the diabetic condition on sugar contents and enzyme activities concerned in the sorbitol pathway in the rabbit lens and of the characteristics of rabbit lens aldose reductase. The levels of aldose reductase, sorbitol dehydrogenase and various sugars were determined in the lenses of normal and experimentally diabetic rabbits.

Experimental

Materials———Rabbits weighing 2—3 kg were used in all the experiments Diabetes was induced in the rabbits by intravenous adminitration of alloxan in isotonic sodium chloride solution, in a single dose of 100 mg per kg. Plasma glucose levels of alloxan-treated rabbits were followed at frequent intervals, and only the animals which showed stable hyperglycemia were used in the study. They were killed approximately 10 weeks after the initial injection of alloxan, and their blood sugar values ranged from 450 to 650 mg/dl. Alloxan-treated rabbits which had been in the diabetic condition for 10 weeks lost about 5—25% of the body weight, and no turbidity of the lens could be seen with the naked eye. Glucose oxidase [EC 1.1.3.4] from *Aspergillus niger*, peroxidase [EC 1.11.1.7] from horseradish, sorbitol dehydrogenase [EC 1.1.1.14] from sheep liver and *myo*-inositol dehydrogenase [EC 1.1.1.18] from *Aerobacter aerogenes* were purchased from Sigma Chemicals.

Nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemicals.

Tissue Preparation——Lenses from normal and diabetic rabbits were removed immediately after the animals had been killed by air embolism, weighed and kept on ice separately. The wet weights of normal and diabetic rabbit lenses were 540.4±56.7 mg ($n=16$) and 519.6±70.5 mg ($n=14$), respectively; the difference between the two values is not statistically significant at the 5% level. For determination of enzymatic activities and sugar contents in the lenses, the tissues were homogenized in 20 mM sodium phosphate buffer (pH 6.8) containing 2 mM dithiothreitol (DTT)(designated as buffer A) at 0—4°C. For studies of the distribution of enzymatic activities in the regions of the lens, the capsule and epithelium of a lens were first removed, and the remaining material was then dissected into cortex and nucleus. The capsule-epithelium and cortex were combined and the combined segments were designated as the outer zone. The outer zone was homogenized in 2 ml of buffer A. The nucleus, designated as the inner zone, was homogenized in 2 ml of the same buffer. Homogenates were centrifuged at 41000×*g* for 45 min at 4°C, and the supernatants were assayed for aldose reductase and sorbitol dehydrogenase activities. For the determination of sugars in the tissue, the homogenate was heated at 70°C for 2 min and centrifuged at 41000×*g* for 30 min. The supernatant was applied to an Amicon Micro-partition System, MPS-1, to obtain a protein-free ultrafiltrate which was assayed for sugar content.

Assay of Aldose Reductase and Sorbitol Dehydrogenase Activities——Aldose reductase activity was determined spectrophotometrically by measuring the decrease in absorption of NADPH at 340 nm with a Union High-Sens SM-401 spectrophotometer equipped with a National X-Y recorder at 25°C. The assay mixture contained 100 mM sodium phosphate buffer (pH 6.4), 10 mM DL-glyceraldehyde or 300 mM D-glucose, 0.15 mM NADPH and an appropriate amount of the sample in a final volume of 3.0 ml. The reaction was initiated by adding the sample, and the decrease of absorption at 340 nm was followed for 200—400 s. Sorbitol dehydrogenase activity was determined in the same manner as aldose reductase activity except that 10 mM D-fructose was used as the substrate and 0.15 mM NADH as the coenzyme. One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol of NAD(P)H per min under the assay conditions described above.

Estimation of Sugar and Sugar Alcohol Contents——Sugar and sugar alcohol contents were determined by enzymatic analysis and gas-liquid chromatography. The enzymatic analyses of glucose[5a] and sorbitol[5b] were done according to the method of Bergmeyer *et al.* *myo*-Inositol was determined according to the method of Weissbach.[6] Gas-liquid chromatography was carried out in the following manner. Aliquots of the protein-free ultrafiltrate obtained by the procedure described above were transferred to test tubes and lyophilized. The contents of the test tubes were further dried in a vacuum desiccator and stored until required. The free sugars and sugar alcohols present in the dried extracts were converted to their trimethylsilyl derivatives by the method of Sweeley *et al.*[7] Sample volumes ranging from 0.5 to 1.5 μl were chromatographed using a Shimadzu GC-4BM gas chromatograph. A glass column (3 mm×1 m) containing Chromosorb WAW (60—80 mesh) as a solid support and 3% SE-30 as a stationary phase was employed. Purified nitrogen gas at a flow rate of 50 ml per min was used as a carrier gas. The oven temperature was 180°C. Quantitation was done by comparing the areas under the curve of compounds in the unknown sample with those of a standard.

Results

Levels of Sugars and Sugar Alcohols in Rabbit Lens

Figure 1 indicates the relative amounts of sugars and sugar alcohols present in the lenses of normal and alloxan-diabetic rabbits on a gas-liquid chromatogram. Low levels of sugars and sugar alcohols were present in the normal lens. In diabetic lens, the contents of sorbitol and fructose were increased and that of *myo*-inositol was greatly decreased. Even in the diabetic condition where an excessive amount of serum glucose was present, a high glucose level was not observed in the lens.

Data on the concentrations of sugars and sugar alcohols in the normal and diabetic rabbit lenses as determined by enzymatic and gas-liquid chromatographic methods are summarized in Table I. In normal lens, the levels of sorbitol and *myo*-inositol were almost the same and about 25 times higher than those of glucose and fructose. On the other hand, significant increases of glucose, sorbitol and fructose, and a considerable decrease of *myo*-inositol were found in diabetic lens. The concentrations of glucose, sorbitol and fructose in the lens are increased by the diabetic condition to about 2, 5 and 10 times normal, respectively, and more than 95% of *myo*-inositol disappeared from the lens under the diabetic condition.

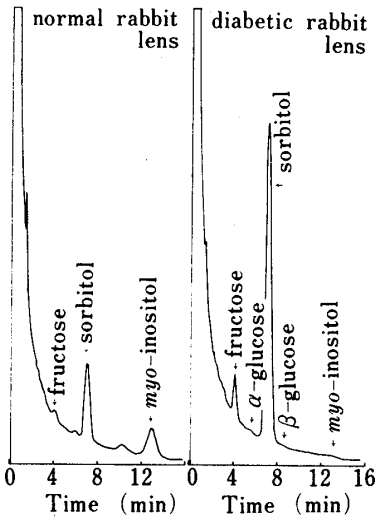

Fig. 1. Gas-Liquid Chromatograph Plots of Sugars present in Normal and Alloxan-Diabetic Rabbit Lenses

Aldose Reductase and Sorbitol Dehydrogenase Activities in Rabbit Lens

Data on aldose reductase and sorbitol dehydrogenase activities in the lenses of normal and diabetic rabbits are summarized in Table II. The activity of aldose reductase in normal lens was about 700 or 276 m units per g wet weight of lens with DL-glyceraldehyde or D-glucose as a substrate, respectively. Sorbitol dehydrogenase activity was about 22 m units per g wet weight of lens, and its activity was very low compared to aldose reductase activity. The activities of both aldose reductase and sorbitol dehydrogenase in diabetic lens seemed to be slightly lower than those in normal lens. However, the differences could not be regarded as significant on the basis of 5% probability of error.

The lens was dissected into two segments, outer zone and inner zone, and the aldose reductase activities in both segments were measured in normal and diabetic lenses (Table III). In normal lens, about 87% of the enzyme activity with DL-glyceraldehyde as a substrate existed in the outer zone and 13% in the inner zone. The proportions of the enzyme activity with D-glucose as a substrate in the outer zone and inner zone were about 79% and 21%, respectively. On the other hand, the enzyme activity with DL-glyceraldehyde as a substrate in the diabetic lens existed in the outer zone and inner zone in proportions of about 93% and 7%, and that with D-glucose as a substrate in proportions of about 95% and 5%, respcetively.

Discussion

Since the first demonstration by Hers[10] that placenta and seminal vesicles contain an enzyme that catalyzes the reduction of a variety of aldoses to the corresponding sugar alcohols, the enzyme aldose reductase has been shown to be widely distributed in mammalian tissues.[8-11] Aldose reductase in the lens was first described by van Heyningen,[1] and it has been suggested that the lens aldose reductase plays a leading role in the etiology of diabetic cataract. An accumulation of glucose, sorbitol and fructose in the lenses of diabetic humans[4] and animals[3,12] has been reported. In this study, alloxan-treated rabbits which had been in the diabetic condition for 10 weeks showed blood sugar levels ranging from 450 to 650 mg/dl, and the contents of sorbitol and fructose in the lens were greatly elevated relative to those in normal lens. The concentration of fructose was less than that of sorbitol. The glucose level was also higher than normal but was much lower than the sorbitol and fructose levels. The low concentration of glucose in diabetic lens seems to be due to the conversion of most of the glucose to sorbitol. *myo*-Inositol is also present in normal lens at the same level as sorbitol. However, in diabetic lens, a significant decrease in *myo*-inositol level was observed. After 10 weeks of the diabetic condition, about 95% of *myo*-inositol had disappeared from the lens. This result is in agreement with the finding of Broekhuyse[13] concerning the change in *myo*-inositol permeability in rat lens due to the sugar cataractous condition. Therefore, the osmotic swelling due to the accumulation of sorbitol may lead to a reduced uptake and an increased efflux of *myo*-inositol, resulting in a low *myo*-inositol level in the lens.

Two enzymes constituting the sorbitol pathway, aldose reductase and sorbitol dehydrogenase, are involved in the formation of sorbitol and fructose in the lens,[1b] and it has been

TABLE I. Sugar Contents of Lenses from Normal and Alloxan-Diabetic Rabbits

Lens	Sugar content (μmol/g wet weight of lens)			
	Glucose	Sorbitol	Fructose	*myo*-Inositol
Normal				
EA	0.231±0.121(12)	6.93±1.55(14)		6.31 ±1.56(14)
GLC		6.11±2.60(9)	0.286±0.084(9)	5.63 ±1.12(9)
Diabetic				
EA	0.456±0.177(9)	28.51±2.62(9)		0.238±0.066(9)
GLC		29.21±2.63(7)	3.06±0.77(7)	

The numbers in parentheses indicate the numbers of lenses studied. EA and GLC indicate enzymatic analysis and gas–liquid chromatography, respectively. The values was means±S.D.

TABLE II. Activities of Aldose Reductase and Sorbitol Dehydrogenase in Lenses from Normal and Alloxan-Diabetic Rabbits

Lens	Enzyme activity (m units/g wet weight of lens)		
	Aldose reductase		Sorbitol dehydrogenase
	DL-Glyceraldehyde	D-Glucose	
Normal	700±81(12)	276±37(13)	22.4±6.4(14)
Diabetic	677±45(8)	249±27(8)	16.9±4.5(7)

The numbers in parenthese indicate the numbers of lenses studied.
The values are means±S.D.

reported by Varma and Kinoshita[3] that in diabetic rat lens the activity of aldose reductase is increased while that of sorbitol dehydrogenase is decreased. In the diabetic rat lens the increase in aldose reductase activity might be expected to accelerate the formation of sorbitol, and moreover, the decrease in sorbitol dehydrogenase activity will also facilitate the accumulation of sorbitol. In rabbit lens, however, even though the levels of aldose reductase and sorbitol dehydrogenase were little affected by the diabetic condition, a significant increase of sorbitol level was observed in diabetic lens. It is considered from this result that the accumulation of sorbitol in diabetic rabbit lens is not directly attributable to changes in the levels of the enzymes concerned with the sorbitol pathway. The apparent K_m value, 135 mM, for glucose of aldose reductase in normal rabbit lens is very large.[14] Consequently, little sorbitol may be formed at normal tissue glucose concentration, but, as a large amount of glucose is incorporated into the lens in the diabetic condition, the enzyme may act on the incorporated glucose to form sorbitol. On the other hand, the sorbitol dehydrogenase level in rabbit lens is lower, being equivalent to about one-thirtieth or one-tenth of the aldose reductase activity with DL-glyceraldehyde or D-glucose as a substrate. Therefore, only a small amount of the sorbitol formed may be converted into fructose, and thus the low level of sorbitol dehydrogenase may also contribute to the accumulation of sorbitol.

Aldose reductase activity in the inner zone of the lens of normal rabbits was 13% of the activity of whole lens with DL-glyceraldehyde as a substrate and 21% of that with D-glucose as a substrate. However, in diabetic rabbit lens, the enzyme activities with DL-glyceraldehyde and D-glucose as substrates in the inner zone were only 7 and 5% of whole lens aldose reductase activity, respectively. The reason for the difference between the proportions of aldose reductase activity in the inner zone in normal and diabetic rabbit lenses is not clear at present. Further work is in progress.

TABLE III. Distribution of Aldose Reductase Activity in Rabbit Lens

Lens	Substrate	Aldose reductase activity (%)	
		Outer zone	Inner zone
Normal	DL-Glyceraldehyde	87.2	12.8
	D-Glucose	78.8	21.2
Diabetic	DL-Glyceraldehyde	93.2	6.8
	D-Glucose	95.2	4.8

The values are the averages of four preparations.

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