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Effects of Some Coumarin Derivatives on the Bovine Lens Aldose Reductase Activity

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Abstract □ Nine coumarin derivatives were examined for their inhibitory effects on bovine lens aldose reductase activity (bovine-LAR). More than 50% inhibition of BLAR activities was observed in the cases of treatments with decursin, decursinol, esculin, isoimperatorin, oxypeucedanine and isobergapten at the concentration of 10^{-4}M. Especially, BLAR activity was completely inhibited by the treatment of decursin and decursinol at this concentration. At 10^{-5}M, only three coumarins-decursin, decursinol and isoimperatorin, were found to have still relatively higher inhibitory effect.

Keywords □ Lens aldose reductase, coumarin derivatives

The tissues bearing the brunt of diabetic manifestations (lens, nerve, kidney, blood vessels and islet cells) are freely permeable to glucose and do not require insulin for glucose penetration as do muscle and adipose tissues, and hence are exposed to the ambient blood glucose levels.

Aldose reductase catalyzes the conversion of free glucose to its sugar alcohol, sorbitol. It possesses broad substrate specificity for many aldoses, and is characterized by low affinity for glucose and galactose[1-7]. The low affinity for hexoses and the availability of excess amounts of free hexoses in diabetes and glalactosemia cause increased formation of the sugar alcohols, sorbitol and galactitol, respectively.

Aldose reductase requires NADPH for its activity, and since NADPH is provided in the cell primariliy by the hexose monophosphate shunt. Aldose reductase appears to be highly localized to certain cell types within tissues. For instance, the highest concentration of aldose reductase is present in the lens epithelium, while it is entirely localized to the Schwann cell in peripheral nerve, to the kidney papilla and the islets of Langerhans in pancreas.

Although insulin deficiency can be ameliorated by diet, insulin injection, or oral hypoglycemic agent therapy, standard treatment has not prevented the development of chronic complications affecting the eyes, kidneys, nerves and arteries[8].

Elevated amounts of glucose in the diabetic lens would result in an intracellular accumulation of sorbitol, because polyols do not readily diffuse across and membrane[9-12].

It is proposed that intracellular polyol accumulation causes osmotic swelling and eventual disruption of cell architecture.

The strongest evidence in favor of the hypothesis is the finding that several drugs that inhibit aldose reductase significantly retard cataract formation in diabetic and galactocemic rats[13].

A number of specific inhibitors of aldose reductase have been described in the literature and they are important in clearing the relationship of sorbitol accumulation to the formation of diabetic and galactocemic manifestations, and may be of possible future clinical use[14-16].

It is still worthwhile trial to find out LAR inhibitors from the natural sources and synthetic preparations. Until now, the influence of coumarine derivatives on the LAR activities has been rarely investigated. In the intensive search of potent LAR inhibitors, we took some coumarine derivatives into the investigation.

MATERIALS AND METHODS

Materials

Bovine eyes were obtained from a local abattoir soon after slaughtering and the lenses were removed and frozen until used. Cellophane seamless tubing 18/32 inch for dialysis was purchased from Wako Chem. Co. NADPH and D,L-glyceraldehyde were purchased from Sigma Co. Coumarin derivatives were gifted from Dr. D.S. Yook of Kyung Hee University.

Preparation of enzyme

All the operations were performed in a cold room at 4 °C. Lenses (60g) were homogenized in 300 m*l* of cold 5 mM phosphate buffer, pH 7.4 and centrifuged at 18,000 g for 15 minutes to remove insoluble materials. 40% ammonium sulfate was added to the supernatant. After the thick suspension had been allowed to stand with occasional stirring for 15 minutes to ensure completeness of precipitation, it was centrifuged and the precipitate was discarded. Aldose reductase was then precipitated from the 40% supernatant solution by the addition of ammonium sulfate to 75% saturation and was recovered by centrifugation. The precipitated enzyme was redissolved in 5 mM phosphate buffer, and dialyzed 3×4 hours against 10 volumes of phosphate buffer. A DEAE-cellulose column (2×30 cm) was previously equilibriated with 5 mM phosphate buffer. The dialyzed enzyme preparation was absorbed on the column, and the column was washed with 5 mM phosphate buffer until the absorbance of effluent at 280 nm was almost zero. Then it was developed with a 0.005-0.150 M linear phosphate buffer gradient (pH 7.4; flow rate, 42 m*l*/hour). The volume of each fraction was 10 m*l* and 70th-100th fractions were collected and used as enzyme source (Fig. 1).

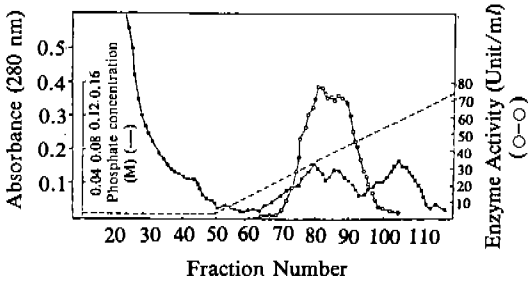

Fig. 1. Fractionation of Lens Aldose Reductase by DEAE-Cellulose Column Chromatography. Protein (□) and enzyme activity (○) were measured. Phosphate buffer (---).

Determination of enzyme activities

The reaction mixture contained 0.1 M phosphate buffer, pH 6.2; NADPH, 2.5×10^{-4}M; D,L-glyceraldehyde, 1.5×10^{-3}M and the enzyme (1). The total volume of this reaction mixture was adjusted to 1 m*l*. The reference blank consisted of all the above compounds except the substrate. The effect of inhibitors on the enzyme activities was determined by including in the reaction mixture the compounds being tested at the desired concentrations. The reaction was carried out at 25 °C and initiated by the addition of substrate. A unit of activity was defined as a change in absorbance of 0.001 unit per minute.

RESULTS AND DISCUSSION

rdfAldose reductase plays a primary role in the promotion of cataractous process in diabetes. Several efforts have been reported to seek specific inhibitors that can control the enzyme activities so that the cataract formation can be prevented or at least delayed. Flavonoids have been most intensively examined for their lens aldose reductase (LAR) inhibitory effects. But only limited investigations have been reported on the LAR inhibitory effects of coumarin derivatives. Therefore, we have conducted the screening test for coumarin derivatives as aldose reductase inhibitors, which have not yet been examined.

In this publication, we present the results of the

Table I. Inhibition Percentage of Bovine Lens Aldose Reductase at 10^{-4}M Coumarins after 2, 5, 8 min. Incubation.

Inhibitors[a]	Incubation Time (%) 2 min.	5 min.	8 min.	Average (%)[b]
Decursin	100	100	100	100
Decursinol	100	100	100	100
Esculin	78	79	80	79
Isoimperatorin	75	76	76	76
Oxypeucedanine	60	61	64	61
Isobergapten	57	58	56	57
Isopimpinellin	48	49	49	49
Pimpinellin	45	46	45	45
Coumarin	30	31	33	31

[a]; Each compound was tested four times and the deviation was less than 5%.

[b]; Average (%) is the mean of the percentage in 2, 5, 8 min.

Table II. Inhibition Percentage of Bovine Lens Aldose Reductase at 10^{-5}M Coumarins after 2, 5, 8 min. Incubation.

Inhibitors[a]	Incubation Time (%) 2 min.	5 min.	8 min.	Average (%)[b]
Decursin	69	68	68	68
Decursinol	59	59	60	59
Esculin	16	15	14	15
Isoimperatorin	54	53	50	53
Oxypeucedanine	33	32	31	32
Isobergapten	27	26	27	27
Isopimpinellin	24	21	25	23
Pimpinellin	22	20	21	22
Coumarin	21	20	18	20

[a]; Each compounds was tested four times and the deviation was less than 5%.
[b]; Average (%) is the mean of the percentage in 2, 5, 8 min.

Table III. Inhibition Percentage of Bovine Lens Aldose Reductase at 10^{-6} M Coumarins after 2, 5, 8 min. Incubation.

Inhibitors[a]	Incubation Time (%) 2 min.	5 min.	8 min.	Average (%)[b]
Decursin	26	25	24	25
Decursinol	17	16	15	16
Esculin	7	6	5	6
Isoimperatonin	18	18	17	18
Oxypeucedanine	14	13	12	13
Isobergapten	20	19	19	19
Isopimpinellin	15	15	14	15
Pimpinellin	14	14	13	14
Coumarin	0	0	0	0

[a]; Each Percentage was tested four times and the deviation was less than 5%.
[b]; Average (%) is the mean of the percentage in 2, 5, 8 min.

investigation on eight coumarin derivatives isolated from *Angelica gigas* (Decursin, Decursinol), *Angelica koreana* (Isopimpinellin), *Heracleum morendorfi* (Isobergaptene, Pimpinellin). Coumarin was taken into the experiment as a reference substance. Coumarin derivatives tested in this experiment showed relatively higher bovine LAR inhibitory effects than the previously reported coumarins in the literature.

Decursin, decursinol, esculin, isoimperatorin, oxypeucedanine and isobergapten inhibited bovine LAR by more than 50% at the concentration of 10^{-4}M, in which two coumarins, decursin and decursinol showed the complete inhibition. At the concentration of 10^{-5}M (Table II), only three coumarins exceeded 50% bovine LAR inhibition, which are decursin, decursinol and isoimperatorin. The most dramatic fluctuation in bovine LAR inhibitory effect was observed in the case of esculsin (Table I and II). Coumarin derivatives of 10^{-6}M (Table III) exhibited the merely faint inhibitory effects on the bovine LAR. Bovine LAR inhibitory effects of esculsin was strongly dose related.

In general, coumarins tested are far less potent in the bovine LAR inhibition than flavonoids reported in the literatures. Even though inhibitory effects of coumarin derivatives tested on the bovine LAR are not sufficient for medical application as a monocomponent preparations, the results might serve as basic data for the study on the additive and synergic effects of coumarin derivatives in edible and medicinal plants and on the applicability of herbs containing coumarin derivatives for the prevention and delaying of diabetic cataract.

Further investigations of LAR inhibitory effects of other coumarin derivatives are also stimulated by this experiment.

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