INHIBITION OF HUMAN PLACENTA ALDOSE REDUCTASE BY TANNIC ACID

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Tannic acid was found to be a highly potent inhibitor of human placenta aldose reductase. The most potent inhibitory component of the tannic acid was isolated and identified as penta-O-galloyl-β-D-glucose, which showed an IC_{50} value of 70 nM. The inhibition by the gallotannin was reversible and of mixed type with respect to DL-glyceraldehyde as the varied substrate.

KEYWORDS aldose reductase; human placenta; tannic acid; gallotannin; inhibition

Aldose reductase (alditol:$NADP^+$ oxidoreductase, EC 1.1.1.21), the promoter of the polyol pathway, leads to sorbitol accumulation in hyperglycemia conditions. The role of the enzyme in the formation of sugar cataract and peripheral neuropathies has been established because aldose reductase inhibitors, such as sorbinil and alrestatin, prevent or significantly delay the onset of diabetic complications in diabetic and galactosemic animals.[1] Several aldose reductase inhibitors can also act as antioxidants.[2] For example, flavonoids, the most ubiqiutously distributed compounds in the plant kingdom, are effective inhibitors of aldose reductase[3] and are also good antioxidants in lipid-peroxidation systems.[4] Recently, some hydrolyzable tannins have been shown to have high inhibitory effects on the *in vitro* lipid peroxidation.[5] Therefore, we examined the inhibitory activity of commercial tannic acid and the extracts from medicinal plants containing the tannins on human aldose reductase, and found that they all inhibited aldose reductase. This communication reports the identification of the most potent inhibitory component of the tannic acid as penta-O-galloyl-β-D-glucose and the steady state kinetics of the aldose reductase inhibition by the gallotannin.

1,3,4,6-Tetra-O-galloyl-β-D-glucose and 1,2,3,4,6-penta-O-galloyl-β-D-glucose were kindly supplied by Prof. I. Nishioka, Kyushu University, Japan.

Aldose reductase activity was spectrophotometrically assayed by recording the oxidation of NADPH at 340 nm. The reaction mixture contained, in a total volume of 2.0 ml, 80 mM potassium phosphate, pH 6.0, 0.1 mM NADPH, 1.5 mM DL-glyceraldehyde, and enzyme. One unit of enzyme was defined as the amount that catalyzed the oxidation of 1 μmole of NADPH/min at 25°C. Tannic acid and its components were dissolved in methanol and 50-μl portions were added to the reaction mixture before the reaction was started by the addition of the enzyme solution. Appropriate blanks were run to correct for nonspecific oxidation of NADPH and absorption by the compounds tested. Kinetic assays were performed similarly with the exception that the concentrations of the substrates and inhibitors were varied. The IC_{50} is the concentration of inhibitor required to inhibit enzyme activity by 50%, and was determined as described.[6]

Aldose reductase from human placenta was purified according to the method of Matsuura et al.[7] The 0.40-0.65 ammonium sulfate fraction of the human placenta extract was dialyzed against 5 mM potassium phosphate buffer, pH 7.4, containing 5 mM 2-mercaptoethanol (Buffer A), and applied to a 2.5 x 50 cm DEAE-Sepharose column equilibrated with the buffer, then the enzyme was eluted with a linear 0-0.12 M

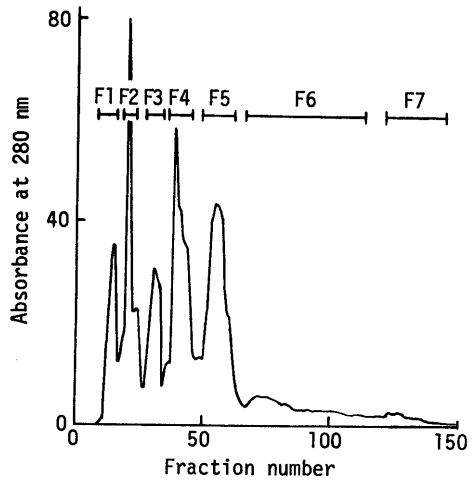

Fig. 1. Elution Pattern of Components in Commercial Tannic Acid on Sephadex LH-20 Chromatography

Table I. Inhibition of Human Placenta Aldose Reductase by Tannic Acid and Its Components Separated on Sephadex LH-20 Chromatography

Compound	IC_{50} (μg/ml)	Yield (mg)
Tannic acid	1.8	
F1	120	67
F2	26	45
F3	2.6	26
F4	0.78	42
F5	0.30	47
F6	0.90	17
F7	2.0	1.2

NaCl gradient in Buffer A. The aldose reductase fractions were pooled, adsorbed on a 1.2 x 5 cm Matrex Red A column in Buffer A, then eluted with the buffer containing 0.5 mM NADPH. The enzyme fractions were concentrated by ultrafiltration with an Amicon YM-10 membrane and passed through a 2.5 x 90 cm Sephadex G-100 column in Buffer A. The enzyme fractions were applied to a 0.8 x 10 cm HA-Ultrogel column equilibrated with Buffer A. The enzyme was eluted out from the column by washing with Buffer A. The specific activity of the purified aldose reductase was 2.1 units/mg.

The components of commercial tannic acid were separated in methanol by chromatography on a 2 x 80 cm Sephadex LH-20 column. Tannic acid (250 mg) was applied to the column and 5 ml-fractions were collected. The elution of gallic acid and gallotannins was monitored by measuring at 280 nm. Reverse phase high performance liquid chromatography (HPLC) was performed by the method of Nishizawa et al.[8].

Tannic acid at relatively low concentrations inhibited human placenta aldose reductase. Commercial tannic acid is a mixture consisting mainly of gallic acid, digallic acid, and gallotannins.[9] The Sephadex LH-20 chromatography of tannic acid resulted in resolution of at least seven components (F1-F7) (Fig. 1). Of the fractions, F4 and F5 exhibited the lowest IC_{50} value for aldose reductase (Table I). Two gallotannins in the fractions were purifed by repeated chromatography on Sephadex LH-20. In the HPLC analysis, the retention times of the two gallotannins purified from F4 and F5 showed single peaks which were coincident with those of authentic 1,3,4,6-tetra-O-galloyl-β-D-glucose and 1,2,3,4,6-penta-O-galloyl-β-D-glucose, respectively. The structures of the two gallotannins were confirmed by comparison of ^{13}C and ^{1}H NMR spectra with those of authentic samples.[8]

The IC_{50} values of tetra-O-galloylglucose and penta-O-galloylglucose were determined to be 0.63 μg/ml (799 nM) and 0.06 μg/ml (70 nM), respectively. The inhibitory potency of the two gallotannins surpasses or is comparable to that of known aldose reductase inhibitors such as sorbinil, quercitrin, and alrestatin: their IC_{50} values have been reported to range from 0.1 to 41 μM.[10]

The inhibition of aldose reductase by penta-O-galloylglucose was reversible; diluting the gallotannin (1 μM)-enzyme mixture to 20 nM concentration with buffer resulted in a recovery of 77% of the native enzyme activity, and 27% of the enzyme activity was inhibited when 20 nM penta-O-galloylglucose was added to the assay mixture directly. This suggests that the inhibition by the gallotannin may not be due to its nonspecific irreversible binding to proteins. The inhibition by penta-O-galloylglucose was of mixed type with respect to DL-glyceraldehyde (Fig. 2). The replots of the slopes and intercepts from the double-reciprocal plots against the concentrations of the gallotannin were linear, which indicates that the gallotannin binds to a specific site on the enzyme.

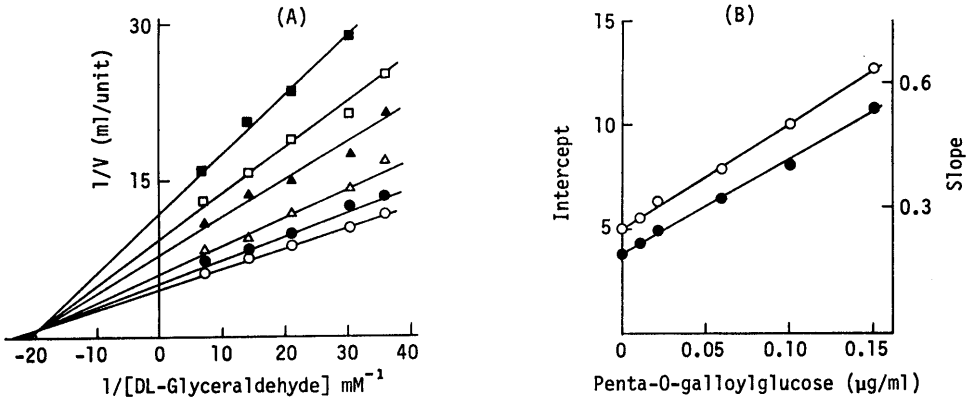

Fig. 2. Inhibition of Aldose Reductase by Penta-O-galloylglucose
(A), Double-reciprocal plots. (B), Secondary plots of the slopes (●) and intercepts (○) from the reciplocal plots versus the concentrations of the gallotannin: 0 (○), 0.01 (●), 0.02 (△), 0.06 (▲), 0.10 (□), and 0.15 (■) μg/ml.

It has been reported that aldose reductase in the ammonium sulfate fraction of the human placenta extract is less susceptible to inhibition by several inhibitors than the enzymes from rat and human lens.[10] However, the human placental enzyme, prepared by further purification, shows sensitivity to the inhibitors similar to that of the lens enzymes.[11] Therefore, placenta is a convenient source of human aldose reductase for conducting initial tests of aldose reductase inhibitors. Penta-O-galloylglucose also potently inhibited aldose reductase from pig lens which has biochemical properties similar to human lens aldose reductase.[12] In contrast, the IC_{50} values of the gallotannin for other pyridine nucleotide dependent oxidoreductases such as human liver aldehyde reductase, human liver carbonyl reductase, and horse liver alcohol dehydrogenase were higher than 1 μM. The relatively specific and potent inhibition of aldose reductase by the gallotannin calls for further studies of its effect in experimental diabetic animals which may provide useful information for controlling diabetic complications.

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