NOTES

Thermorubin and 2-Hydroxyphenyl Acetic Acid, Aldose Reductase Inhibitors

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Aldose reductase (EC 1.1.1.21) catalyzes the conversion of glucose to sorbitol and promotes the accumulation of sorbitol in various tissues under the condition of hyperglycemia such as diabetes mellitus. The accumulated intracellular sorbitol causes diabetic complications such as cataracts,[1] neuropathy,[2] and retinopathy.[3] It has been reported that inhibitors of aldose reductase reduce the tissue sorbitol content in diabetic animals and are useful as therapeutic agents for diabetic complications.[4,5]

In a search for a novel aldose reductase inhibitor from thermophilic microbial metabolites, we found that *Thermoactinomyces* sp. UAT-8 produced thermorubin which exhibits potent inhibitory activity against various aldose reductases (Fig. 1). Thermorubin has been previously isolated as an antibiotic by R. CARAVERI *et al.*,[6] however aldose reductase inhibitory activity of thermorubin has not been reported. During the purification of thermorubin, 2-hydroxyphenyl acetic acid, coproduced in culture broth of the strain UAT-8, was also isolated as an aldose reductase inhibitor. 2-Hydroxyphenyl acetic acid has not been previously reported as aldose reductase inhibitor. This paper deals with the inhibitory activities of thermorubin and 2-hydroxyphenyl acetic acid on aldose reductase and the inhibitory effects of thermorubin on sorbitol accumulation in rat lenses.

Thermorubin and 2-hydroxyphenyl acetic acid were purified from culture broth of *Thermoactinomyces* sp. UAT-8 by silica gel, ODS, and Sephadex LH-20 column chromatographies, and preparative HPLC. The structures of these compounds were confirmed by spectroscopic analyses and direct comparison with authentic samples. The sample of thermorubin was isolated from culture broth of *Thermoactinomyces antibioticus* ATCC 14570.[6]

Aldose reductase inhibitory activity was measured according to the method of HAYMAN and KINOSITA with slight modification.[7] Calf lenses were obtained from a local abattoir within 2 hours after slaughtering. Rat lenses were enucleated from the eyes of male Wister rats. Lenses were homogenized in 3 volumes of cold distilled water and centrifuged at $10,000 \times g$ for 15 minutes to remove insoluble material. The supernatant was dialyzed overnight against 50 mM sodium chloride. This dialyzed lens homogenate was used for enzymatic assay as partially purified aldose reductase. Recombinant human aldose reductase (Wako chemical Co., Ltd.) was used for assay of human aldose reductase activity. Enzymatic reaction was performed in 1 ml of 50 mM sodium phosphate buffer (pH 6.2) containing 0.125 mM NADPH, 400 mM lithium sulfate, enzyme solution, and 3 mM DL-glyceraldehyde as the substrate. The inhibitory effect of test compounds was determined by including 10 μl of inhibitor solution in the reaction mixture. The reaction was initiated with the addition of substrate. The rate of NADPH oxidation was determined spectrophotometrically at 340 nm.

Inhibitory effects of thermorubin on intracellular sorbitol accumulation in rat lens were determined according to methods of TERASHIMA *et al.*[8] The lenses removed from Sparague-Dawley rat (male, 180~200 g) were incubated in a medium equilibrated with air containing 5% CO_2 for 6 hours at 37°C. The medium consisted of 117.6 mM NaCl, 3.78 mM KCl, 0.54 mM $MgSO_4$, 0.27 mM NaH_2PO_4, 0.225 mM KH_2PO_4, 26.1 mM $NaHCO_3$, 0.9 mM $KHCO_3$, 1.25 mM $CaCl_2$, 50 mM glucose and varying amounts of test compounds. Lenses were homogenized in cold 8% perchloric acid and the homogenates were centrifuged. The supernatants were neutralized at 4°C with 2 N KOH. Sorbitol content in the supernatants was determined enzymatically according to the method of BERGMEYER *et al.*[9] Epalrestat (Ono pharmaceutical Co., Ltd., designated as ONO-2235), clinically used aldose reductase inhibitor,[8] was used as a standard sample in the above mentioned assays.

Thermorubin inhibited aldose reductase in a dose-dependent manner and its IC_{50} values were 1.3 nM on rat lens aldose reductase, 150 nM on bovine lens aldose reductase and 54 nM on human aldose reductase, respectively. Under the same condition, IC_{50} values of epalrestat were 2.1 nM on rat lens aldose reductase, 40 nM on bovine lens aldose reductase and 37 nM on human

Fig. 1. Structure of thermorubin.

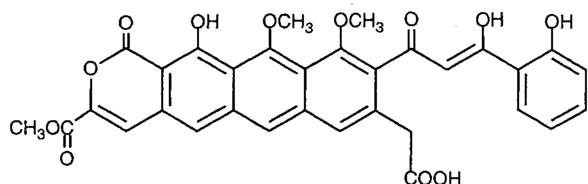
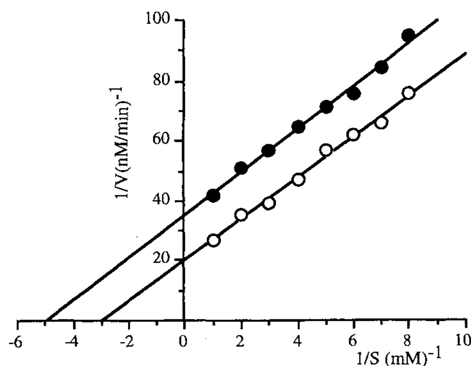
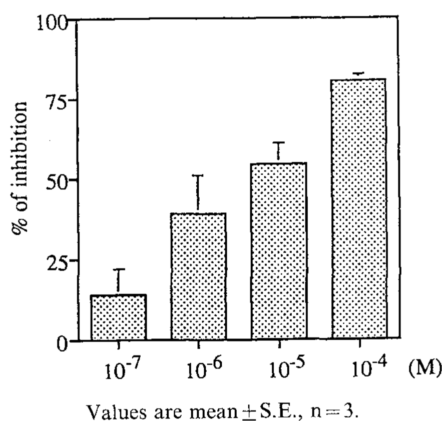


Fig. 2. Lineweaver-Burk plot for the inhibition of human aldose reductase by thermorubin.



Human aldose reductase activity was measured with varying concentrations of DL-glyceraldehyde in the presence of thermorubin at concentrations of 0 (○) and 40 nM (●).

Fig. 3. Inhibition of sorbitol accumulation in the isolated rat lenses by thermorubin.



Values are mean ± S.E., n = 3.

aldose reductase, respectively. Thermorubin also inhibited rat lens aldose reductase with an IC_{50} of 8 nM using 890 mM glucose as substrate. IC_{50} value of epalrestat was 14 nM on rat lens aldose reductase using glucose as substrate. Thermorubin did not inhibit other reductase such as lactate dehydrogenase and glucose-6-phosphate dehydrogenase. Therefore, it appears that thermorubin is very specific against aldose reductases. An enzyme kinetic study of thermorubin was performed using a Lineweaver-Burk plot for human aldose reductase inhibition. Fig. 2 shows that thermorubin inhibited human aldose reductase uncompetitively with DL-glyceraldehyde as substrate.

Sorbitol content in the lenses was increased after the incubation of isolated rat lenses in the presence of 50 mM glucose. The addition of thermorubin in the incubation medium caused dose-dependent inhibition of sorbitol accumulation in the lens with an IC_{50} value of 7.1 μM (Fig. 3). The IC_{50} value of epalrestat was 3.0 μM under the same condition.

2-Hydroxyphenyl acetic acid inhibited the enzymatic activity of bovine lens aldose reductase and recombinant human aldose reductase with IC_{50} values of 4.6 μM and 2.6 μM, respectively. The inhibitory activities of 2-hydroxyphenyl acetic acid against lactate dehydrogenase and glucose-6-phosphate dehydrogenase were about 5,000-fold less than that against aldose reductase. The kinetic study of 2-hydroxyphenyl acetic acid was made on a Lineweaver-Burk plot for bovine lens aldose reductase inhibition. 2-Hydroxyphenyl acetic acid inhibited bovine lens aldose reductase uncompetitively with DL-glyceraldehyde as substrate.

References

1) KINOSITA, J. H.: Mechanism initiating cataract formation. Proctor Lecture Invest. Ophthalmol. 13: 713 ~ 724, 1974
2) GABBAY, K. H.: The sorbitol pathway and the complications of diabetes. N. Engl. J. Med. 288: 831 ~ 836, 1973
3) KUWABARA, T. & D. G. GOGAN: Retinal vascular patterns VI. Mural cells of the retinal capillaries. Arch. Ophthalmol. 69: 492 ~ 502, 1963
4) PETERSON, M. J.; S. SARGES, C. E. ALDINGER & D. P. MACDONALD: CP-45,634: A novel aldose reductase inhibitor that inhibits polyol pathway activity in diabetic and galactosemic rats. Metabolism 28: 456 ~ 461, 1979
5) DVORNIC, D.; N. SIMARD-DUGUESNE, M. KRAMI, K. SESTANJ, K. H. GABBAY, J. H. KINOSHITA, S. D. VARMA & O. MEROLA: Polyol accumulation in galactosemic and diabetic rats: Control by an aldose reductase inhibitor. Science (Wash, DC) 182: 1146 ~ 1148, 1973
6) CRAVERI, R.; C. CORONELLI, H. PAGANI & P. SENSI: Thermorubin, a new antibiotic from a thermoactinomycete. Clin. Med. 71: 511 ~ 521, 1964
7) HAYMAN, S. & J. H. KINOSITA: Isolation and properties of lens aldose reductase. J. Biol. Chem. 240: 877 ~ 882, 1965
8) TERASIMA, H.; K. HAMA, R. YAMAMOTO, M. TSUBOSHIMA, R. KIKKAWA, I. HATANAKA & Y. SHIGETA: Effects of a new aldose reductase inhibitor on various tissues *in vitro*. J. Pharmacol. Exp. Ther. 229: 226 ~ 230, 1984
9) BERGMEYER, H. U.; W. GRUBER & I. GUTMANN: Methods for determination of metabolites; D-sorbitol. *In* Methods of Enzymatic Analysis, *Ed.*, H. U. BERGMEYER, pp. 1323 ~ 1326, Academic Press, New York, 1974