

Inhibition of Aldose Reductase Activity by Extracts from Hops

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ABSTRACT

J. Inst. Brew. 108(3), 344–347, 2002

The inhibitory effect of beer on aldose reductase was investigated. Components present in beer strongly inhibited aldose reductase. Inhibition activity was observed primarily in the methanol-soluble fraction of the beer and active components were identified to be of hop origin. Further, a component was identified as iso- α -acids, which are well known as main bitter components of beer. The inhibition rate of iso- α -acids was similar to quercetin, which is known to be a strong inhibitor of aldose reductase activity.

Key words: Aldose reductase, hop, α -acids, iso- α -acids.

ABBREVIATIONS

NADPH – nicotinamide adenine dinucleotide phosphate
PBS – phosphate buffered saline

INTRODUCTION

Aldose reductase (EC 1.1.1.21) is a member of the aldo-keto reductase family of NADPH-dependent oxidoreductases⁸. This enzyme is a key enzyme in the polyol pathway. Aldose reductase reduces glucose to sorbitol and is related to many complications associated with diabetes^{2,12}. The polyol pathway has been found in many tissues such as the eye lens and kidney, where diabetic complications are a concern. Sorbitol can be produced more rapidly by aldose reductase than it is converted to fructose by sorbitol dehydrogenase, resulting in an accumulation of sorbitol. The intracellular accumulation of a polar sugar alcohol can produce a hyperosmotic effect, which has been observed to lead to membrane permeability changes and the onset of cellular pathology^{6,7}. The inhibition of aldose reductase is a possible method to prevent or delay diabetic peripheral neuropathy.

Recently, some aldose reductase inhibitors, for example sorbinil, torestat and quercetin were found to be useful for preventing or treating chronic complications caused by diabetes¹⁴. There have been several studies that screened

plants and sea vegetables for inhibitory effects on aldose reductase and various inhibitors were found^{9–11}.

In this study, we examined the inhibition of aldose reductase activity by extracts of hops (*Humulus lupulus* L.) and investigated the effects of the iso- α -acids on aldose reductase activity in detail.

MATERIALS AND METHODS

Chemicals

Recombinant human aldose reductase and 0.01 mol/L phosphate buffered saline (PBS) were obtained from Wako Pure Chemical Co. (Osaka, Japan). α -Acids and iso- α -acids were obtained from Labor Veritas (Zurich, Switzerland). Quercetin was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from the Oriental Yeast Co., and DL-glyceraldehyde from the Wako Pure Chemical Co. (Osaka, Japan). Malt and hops (Hallertau) were obtained from Asahi Beer Malt Co. (Siga, Japan).

Preparation of lyophilised beer and wort

Degassed beer or wort (50 mL) was transferred to 500 mL round-bottom flasks and frozen at -40°C . The sample was then concentrated by lyophilisation.

Laboratory scale mashing

Milled lager malt (304 g) was mixed with 1,000 mL of water. This mash consisted of 0.36 g CaSO_4 and 0.15 g NaCl. The mash was held at 48°C for 20 min and the temperature was raised to 75°C at $1^{\circ}\text{C}/\text{min}$ and held at 75°C for 10 min. The mash was then made up to 1,000 mL with deionised water at 75°C and filtered through fluted filter paper, the first 50 mL of filtrate being recycled.

Extraction of aldose reductase inhibitor

The extracts of lyophilised beer or wort were prepared as follows: samples (100 mg) were transferred into various solutions (4 mL) such as ethanol, methanol, and 0.15 M phosphate-buffered saline (PBS, pH 7.2) in 15 mL Falcon tubes (Becton Dickinson and Company, USA), respectively and shaken at 100 rpm for 1 h at room temperature. Another treatment (BPBS, pH 7.2) involved boiling the PBS sample for 10 min. The suspensions were centrifuged at $12,000 \times g$ for 10 min and the supernatants were used for aldose reductase activity assay.

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Extraction of the iso-octane soluble fraction of beer

The iso-octane soluble fraction of beer was extracted as follows: degassed beer (500 mL) was concentrated to 10 mL by rotary evaporation. The sample (10 mL) was acidified with HCl (6 M; 0.5 mL) and subsequently partitioned with iso-octane (20 mL; Kanto Chemical Co., Inc., Tokyo) with vigorous shaking for 20 min. After phase separation, the iso-octane layer (20 mL) was collected and the solvent was removed under reduced pressure (rotavapor; 30°C). The residue was redissolved in methanol (5 mL; HPLC-grade, Kanto Chemical).

Assay of aldose reductase activity

Aldose reductase activity was assayed spectrophotometrically with a Bio-Rad Model 3550- UV microplate reader using a 96-well microplate. The reaction mixture in a total volume of 100 μ L contained 100 mM sodium phosphate buffer (pH 6.2), 0.15 mM NADPH, 10 mM glyceraldehyde as a substrate, and the enzyme solution. The reaction rate was determined by tracing the decrease in the absorption of NADPH at 340 nm.

The aldose reductase inhibition rate was given by the equation¹⁶

$$\text{Inhibition rate (\%)} = [1 - \Delta A_s - \Delta A_b] / (\Delta A_c - \Delta A_b) \times 100$$

where ΔA_s is the decreased rate of sample absorbance, ΔA_b is the decreased rate of blank absorbance, and ΔA_c is decreased rate of control absorbance.

Analysis of polyphenols

Polyphenols were determined according to Jerumanis with some modifications¹. Instead of 10 mL beer, 10 mL iso-octane soluble fraction was used. After addition of

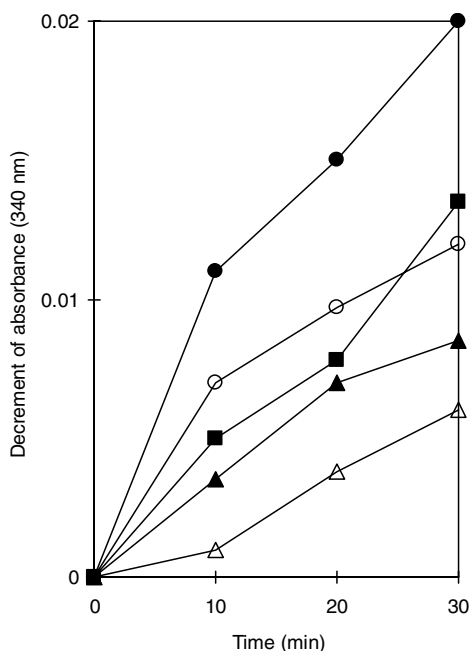


FIG. 1. Inhibition of aldose reductase activity by beer (lyophilised). ● control; ■ PBS-soluble fraction; ▲ ethanol-soluble fraction; △ methanol-soluble fraction; ○ BPBS-soluble fraction.

ferric and the ammonia reagents, the suspensions was centrifuged (10 min, 3000 g) and the absorbance of the aqueous phase measured at 600 nm.

RESULTS

Inhibition of aldose reductase activity by beer

The aldose reductase inhibiting activity of beer was investigated. As shown in Fig. 1, the 340 nm-absorbance was gradually decreased in the reaction mixture without lyophilised beer, *i.e.* aldose reductase activity was not inhibited. However, the decrease in 340 nm-absorbance was lower in the reaction mixture with lyophilised beer compared with that without lyophilised beer. It was suggested that the lyophilised beer inhibited aldose reductase activity. In particular, lyophilised beer dissolved in methanol indicated strong inhibition for aldose reductase activity. These results suggested that the beer contained an inhibitor for aldose reductase activity and that it was ethanol-soluble. Beer has not previously been reported to have inhibitory activity on aldose reductase.

Inhibition of aldose reductase activity by malt or hops

To confirm the effect of the materials of the beer on the inhibition of aldose reductase activity, aldose reductase inhibition by malt or hops was investigated. As shown in Fig. 2, the aldose reductase activity was inhibited by extracts from malt that were prepared without hops. On the other hand, aldose reductase activity was strongly inhibited by wort prepared from malt and hops. The weight of malt in the wort was 150-fold higher compared to the hops. This suggested that strong inhibitor(s) for aldose reductase activity were contained in hops. There have been no studies reported that hops contained aldose reductase inhibitor(s).

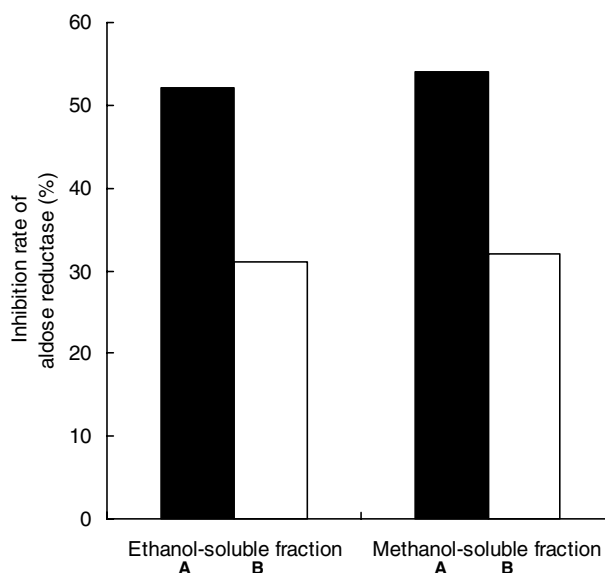


FIG. 2. Inhibition of aldose reductase activity by malt or hops: A, The wort (lyophilised) was initially prepared with malt and hops; B, The wort (lyophilised) was initially prepared with malt.

Inhibition of aldose reductase activity by the iso-octane fraction extracted from beer

To clarify the inhibition of iso- α -acids by those contained in beer, the iso-octane soluble fraction of beer was investigated. The iso- α -acids in beer were easily dissolved

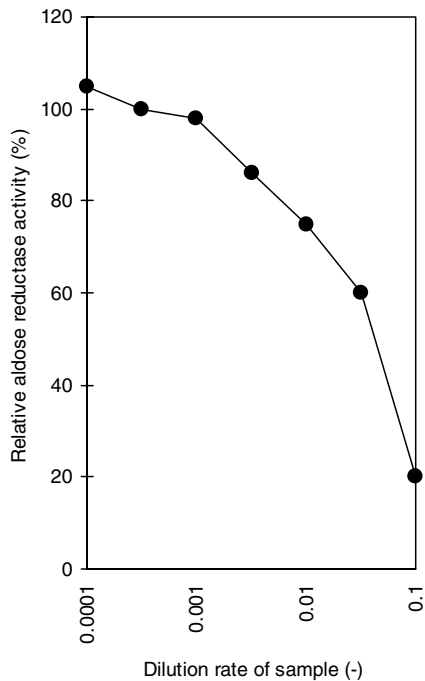


FIG. 3. Inhibition of aldose reductase activity by iso-octane fraction extracted from beer. Prepared sample was diluted with PBS and aldose reductase activity was assayed.

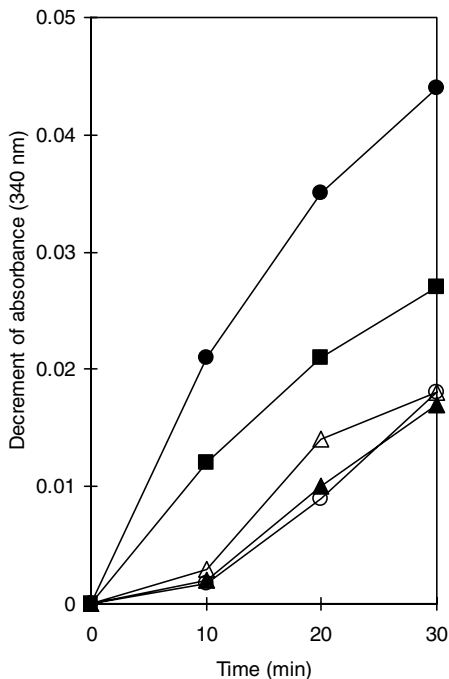


FIG. 4. Inhibition of aldose reductase activity by extractions from hops. ● control; ■ PBS-soluble fraction; ▲ ethanol-soluble fraction; △ methanol-soluble fraction; ○ BPBS-soluble fraction.

in iso-octane²⁰. The iso-octane soluble fraction was evaporated and dissolved in methanol. As shown in Fig. 3, the iso-octane soluble fraction showed extensive inhibition for aldose reductase activity. However, this fraction contained not only iso- α -acids but also other substances such as polyphenols. The polyphenols in the iso-octane soluble fraction were detected (not data shown). The polyphenols and the flavonoids are known aldose reductase inhibitors^{19,22}.

Inhibition of aldose reductase activity by extractions from hops

Inhibition of aldose reductase activity by extraction from hops was investigated. Various solutions (4 mL) such as ethanol, methanol, PBS and BPBS, respectively were added to the hop pellet (100 mg). As shown in Fig. 4, extractions from hops that were treated with boiling in PBS showed strong inhibition of aldose reductase activity. It was suggested that the inhibitors for aldose reductase activity were included in the hop pellets. Furthermore, the composition of inhibitors was converted easily by boiling in PBS. The α -acids in hops are known to be easily converted to iso- α -acids by boiling in wort²⁰. Furthermore, the α -acids dissolved less in water, and the iso- α -acids dissolved more easily in water²⁶.

Inhibition of aldose reductase activity by iso- α -acids

To clarify the effects of iso- α -acids on inhibition of aldose reductase activity, the aldose reductase activity inhibition using purified iso- α -acids was investigated. The standard iso- α -acids are comprised of three components: iso-humulone, iso-cohumulone, and iso-adhumulone. As shown in Fig. 5, the aldose reductase activity was inhibited 48% with 33 μ g/mL of iso- α -acids. When the inhibition rate for aldose reductase activity was investigated using quercetin under the same conditions, the aldose reductase activity was inhibited 52% with 33 μ g/mL of quercetin,

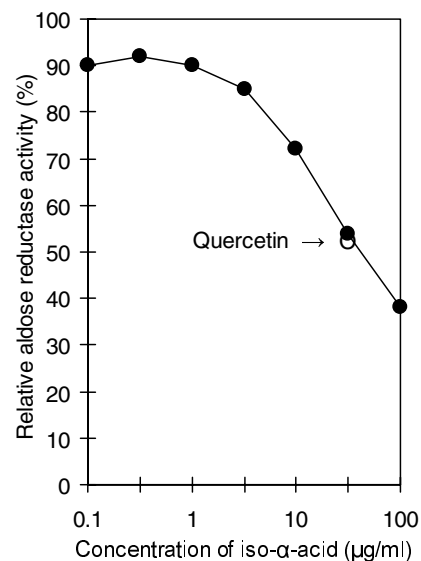


FIG. 5. Inhibition of aldose reductase activity by iso- α -acid. ● iso- α -acid; ○ quercetin.

which is known as a strong aldose reductase inhibitor⁹. Iso- α -acids are suggested to be a potent inhibitor of human aldose reductase similar to quercetin.

DISCUSSION

Aldose reductase is considered to play an important role in the development of diabetic cataracts¹¹, neuropathy⁶, retinopathy^{3,15} and possibly nephropathy⁵. There have been several studies that searched for aldose reductase inhibitors. Some aldose reductase inhibitors are known such as sorbinil, tolrestat and eparestant²⁵. Iwahori *et al.* reported that Chinese crude drugs and marine algate have inhibitory effects on bovine lens aldose reductase¹⁶⁻¹⁸. Kohda *et al.* reported that flavonoid or tannin-containing plants generally have strong inhibitory effect on aldose reductase from rabbit lens¹⁴. Various aldose reductase inhibitors are known to show different efficacies among aldose reductase from various animals. So, it is important that the assay for aldose reductase inhibitory activities uses human aldose reductase. Quercetin is known to be a strong inhibitor of aldose reductase. From the results shown in Fig. 2, the aldose reductase activity was inhibited by extracts from malt that were prepared without hops. Many polyphenols are known to be potent inhibitors of aldose reductase^{8,19,22}. The wort prepared with malt contained 200 mg/L polyphenol. It was suggested that aldose reductase was inhibited by the polyphenols contained in beer. However, the aldose reductase activity was markedly inhibited by wort prepared from malt and hops. These results suggested that hops contained a strong inhibitor of aldose reductase. Furthermore, iso- α -acids showed a strong inhibition of aldose reductase activity as shown in Fig. 5.

From the above discussion, the present results suggest that the iso- α -acids inhibited the aldose reductase activity significantly. Hops are used as a tranquilizer or bitter stomachic in folk medicine. Some of the hops components are bioactive agents. For example, the hop bitter acids (iso- α -acids) have antibiotic and antioxidative activities, and hop cones have been reported to contain some bioactive substances which have an estrogen-like activity or gonadotropin inhibitory activity^{4,13,23}. Recently, Tagashira *et al.* reported that the hop bract polyphenols inhibited the cellular adherence of cariogenic streptococci at much smaller concentrations than the polyphenols extracted from oolong tea or green tea leaves^{23,24}. However, there are no reports about the inhibition of aldose reductase activity. Hop extract was not previously reported to have an inhibitory activity on aldose reductase. Therefore, this is the first study to show that the iso- α -acids showed a significant inhibitory effect on aldose reductase.

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(Manuscript accepted for publication August 2002)