Inhibitory Effect of Heat-Treated 3-(3′,4′-dihydroxyphenyl)-l-alanine (DOPA) on β-glucuronidase Activity

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Received May 20, 2013; Accepted July 1, 2013

β-Glucuronidase may contribute to the development of colon cancer. A reduction in the activity of this enzyme could inhibit the enterohepatic circulation of carcinogens, which is thought to reduce the risk of colon cancer. The purpose of this study was to investigate the effect of heat treatment on the β-glucuronidase inhibition activity of DOPA. Prior to heating, DOPA showed only a weak inhibitory effect on β-glucuronidase. After heating a DOPA solution at 100°C for 10 min, the heat-treated DOPA (hDOPA) exhibited 48.5% inhibition at 10 µM. We performed a kinetic study on β-glucuronidase inhibition by hDOPA using Lineweaver-Burk analysis. The kinetics profile suggests that hDOPA competitively inhibits β-glucuronidase.

Keywords: 3-(3′,4′-dihydroxyphenyl)-l-alanine (DOPA), β-glucuronidase, microbiota

Introduction
The gastrointestinal microbiota plays a key role in certain pathological disorders, including colon cancer and inflammatory bowel diseases (Guarner and Malagelada, 2003). Bacterial enzymes, including β-glucosidase, β-glucuronidase, and β-galactosidase, may contribute to the development of colon cancer (Chadwick et al., 1992). Carcinogens taken into the body are metabolized and detoxified by conjugation, including glucuronidation, in the liver, and then excreted via urine. Some of the conjugates escape excretion and are transferred to the intestinal tract via bile. These conjugates can be degraded by bacterial β-glucuronidase (EC 3.2.1.31), an exoglycosidase enzyme that catalyzes the cleavage of glucuronosyl-O bonds (Khan et al., 2002) and remains in the intestinal tract to liberate potentially carcinogenic compounds (Freeman, 1986; Knasmüller et al., 2001). High fecal β-glucuronidase activities have been associated with an increased risk of colon cancer (Rafter et al., 2004). Furthermore, fecal β-glucuronidase activity in colon cancer patients is significantly higher than in healthy controls (Kim and Jin, 2001). Therefore, reduction of β-glucuronidase activity in the intestinal tract could play an important role in the prevention of colonic carcinogenesis.

It has been shown that using broad-spectrum antibiotics reduces bacterial β-glucuronidase activity in the large intestine (Takasuna et al., 1996; Takasuna et al., 1998; Kehrer et al., 2001). However, using these antibiotics might induce the development of clinically relevant resistance in bacterial strains, which might cause the antibiotics to lose their β-glucuronidase inhibition activities. Furthermore, the intestinal biota plays essential roles in carbohydrate metabolism, vitamin production, and the processing of bile acids, sterols, and xenobiotics (Cummings and Macfarlane, 1997; Guarner and Malagelada, 2003). Thus, the removal of gastrointestinal bacteria for the reduction of β-glucuronidase activity is not recommended.

Diet influences the β-glucuronidase activity of the intestinal microbiota. High-protein and high-fat diets have been reported to be associated with elevated levels of bacterial β-glucuronidase activity in the large intestine of rats (Reddy et al., 1977). It has also been shown that consumption of dietary fiber, such as cellulose (Freeman, 1986), oligosaccharides (Rowland et al., 1998), edible seaweeds (Undaria pinnatifida and Porphyra ternera) (Mallett et al., 1986), and rice bran (Gestel et al., 1994), reduces intestinal β-glucuronidase activity. Modulation of the microbiota also changes the enzyme activity. Consumption of Bifidobacterium adolescentis...
Another way to reduce β-glucuronidase activity is through the use of β-glucuronidase inhibitors. Carcinogen-treated rats fed a diet containing β-glucuronidase inhibitors, such as d-glucaric acid and its metabolite, demonstrated reduced fecal β-glucuronidase activity and experienced a reduced number of chemically induced colon tumors (Takada et al., 1982; Morita et al., 2008).

Several hundred phytochemicals have been identified in foods of plant origin, including fruits, vegetables, whole grain cereals, and legumes. Many of them are served after processing or cooking. Therefore, we investigated the effect of heat-treated phytochemicals on β-glucuronidase activity. During random screening of phytochemicals, we found that heat-treated DOPA (hDOPA) can inhibit β-glucuronidase activity. The purpose of this study was to investigate the effect of heat treatment of DOPA on its β-glucuronidase-inhibitory efficacy.

Materials and Methods

Chemicals β-Glucuronidase from E. coli, p-nitrophenyl-β-d-glucuronide (PNPG), and DOPA were purchased from Sigma-Aldrich Japan (Tokyo, Japan).

Heat treatment One milliliter of 150 µM DOPA in 20 mM phosphate buffer, pH 7.0, was sealed in Pyrex tubes (100 mm length, 16 mm diameter) and heated in an ALB-121 thermo aluminum bath (Scinics, Tokyo, Japan) at 100°C. Tubes were then removed from the thermo aluminum bath (for each time point, three tubes were analyzed using the β-glucuronidase inhibition assay) at different times (5 − 25 min) and immediately cooled in an ice bath.

β-Glucuronidase inhibition assay protocol β-Glucuronidase activity was assayed by monitoring p-nitrophenol production (at absorbance of 405 nm) formed from the substrate (PNPG) according to the method of Gudiel-Urban and Goñi (2002), with minor modification. In brief, 50 µL of DOPA or hDOPA solution in 20 mM phosphate buffer, pH 7.0, and 50 µL of β-glucuronidase (12 units/mL) were added to a 96-well microplate and incubated at 37°C for 10 min. Next, 50 µL of 0.6 mM PNPG were added to each well to start the reaction. The microplate was read on a Sunrise™ microplate reader (Tecan Japan Co., Ltd., Kanagawa, Japan) at 405 nm at 37°C for 5 min. The enzyme activity was expressed as a change in absorbance at 405 nm per min (slope) calculated using Magellan™ software (Tecan Japan Co., Ltd., Kanagawa, Japan). The percentage inhibitory activity was calculated using the formula: \( \left( \frac{E - S}{E} \right) \times 100 \), where \( E \) denotes enzyme activity without the test material and \( S \) denotes enzyme activity with the test material.

HPLC analysis DOPA concentration was determined by HPLC as described by Kubo et al. (2004) with minor modifications. HPLC analysis was performed using a Develosil RPAQUEOUS column (i.d. 4.6 mm × 250 mm; Nomura Chemical Co., Ltd., Aichi, Japan), a PU-1580 pump (JASCO, Tokyo, Japan), and a UV-2070 UV-vis detector (JASCO, Tokyo, Japan). The analysis was performed under isocratic conditions using 20% acetonitrile/ultrapure water containing 0.1% trifluoroacetic acid at a flow rate of 1.0 mL/min, an injection volume of 20 µL, and UV detection at 280 nm. Under these conditions, DOPA eluted with a retention time of 6.3 min.

Statistical analysis Statistical analysis was performed using Tukey’s HSD test.

Results and Discussion

Effect of heat-treated DOPA (hDOPA) on β-glucuronidase activity As shown in Fig. 1, DOPA exhibited a weak inhibitory effect on β-glucuronidase activity prior to heating. After heating the DOPA solution at 100°C, it exhibited a greater inhibitory effect on β-glucuronidase activity. The percentage of β-glucuronidase inhibition increased as the heating time increased, up to 10 min, at which point additional heating did not cause any significant effect. The inhibitory effect of hDOPA at different concentrations is shown in Fig. 2. hDOPA inhibited β-glucuronidase in a dose-dependent manner. DOPA is easily oxidized to DOPA quinone in the presence of water (Fig. 3; Kankkunen et al., 2002). Therefore, we investigated the stability of DOPA during heat treatment. We found that heating the solution reduced the DOPA concentration. After heating at 100°C for 10 min, the DOPA concentration was reduced to 55.0 ± 1.9% (n = 3) of its original value. This result suggests that the heat-induced degradation products of DOPA

![Fig. 1. Effect of heat treatment on the inhibition of β-glucuronidase by DOPA. Results are presented as mean ± SD for triplicate measurements. Significant differences are indicated by different letters.](image-url)
Inhibitors of β-glucuronidase could be used for the prevention of chemical carcinogenesis (Takada et al., 1982; Morita et al., 2008). Furthermore, the inhibitors could alleviate cancer drug toxicity. As in the case of carcinogens, the metabolite of irinotecan, which is commonly used as a chemotherapeutic agent for colon cancer, is glucuronidated in the liver and excreted into the intestine, where bacterial β-glucuronidase reactivates it and causes severe diarrhea. Therefore, β-glucuronidase inhibitors could be effective for reducing the dose-limiting side effect of irinotecan (Wallace et al., 2010).

β-glucuronidase inhibitors also played an important role in lowering the level of neonatal jaundice. Bacterial β-glucuronidase produces unconjugated bilirubin and enhances the enterohepatic circulation of bilirubin. Infants who consume casein hydrolysate formula have been shown to have a lower level of neonatal jaundice than infants who consume routine formula or breast milk. L-Aspartic acid, one component of hydrolyzed casein milk, was identified as a β-glucuronidase inhibitor (Kreamer et al., 2001).

DOPA is one of the major therapeutic agents used for the treatment of Parkinson’s disease (Cotzias et al., 1967). However, a potential role of DOPA in neurotoxicity via oxidative stress has been suggested (Soliman et al., 2002; Chen et al., 2003; Maharaj et al., 2005). The high DOPA content of some kinds of beans renders them less suitable for food and feed uses. The mucuna bean (Mucuna pruriens var. utilis), for example, has high nutritional value. However, it is rarely used as a feed because of its high DOPA content. Dahouda et al.
DOPA could inhibit β-glucuronidase activity. The effect of cooking on the physiological functions of legume- and animal nutrition, also contains DOPA. Its DOPA content safely incorporated in the diet of poultry.

The faba bean (Vicia faba L.), which is one of the oldest crops grown by man, providing high-protein seeds for human and animal nutrition, also contains DOPA. Its DOPA content has been reported to increase during germination (Goyoaga et al., 2008). Legumes have long been used as a food. The effect of cooking on the physiological functions of legume-containing DOPA is therefore of great interest.

The present study demonstrated that heat-processed DOPA could inhibit β-glucuronidase activity. The in vivo effect of hDOPA on bacterial β-glucuronidase activity in the intestine will be the subject of a further study.

References


(2009) showed that heat processing was effective in reducing DOPA concentrations. They reported that cooking (boiling for 30 min) of the mucuna bean reduced the DOPA level by 52%. They also showed that cooked mucuna bean could be safely incorporated in the diet of poultry.


