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Original Article

# Effect of Green Tea on Porcine Intestinal Glutamate Carboxypeptidase II Activity

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## Summary

Food folates exist mainly as pteroylpolyglutamate, which is degraded to a monoglutamyl form by glutamate carboxypeptidase II (GCPII) in the intestine before absorption. Green tea is a traditional and widely consumed drink all over the world and one of the major sources of food folates. In this study, we examined the influence of food, including green tea, on porcine GCPII activity *in vitro*. Several foods were selected and their extracts were prepared in order to examine the influence on GCPII activity. The food extracts were included in the reaction mixture for the analysis of GCPII activity at the level of 10%. Little inhibition of GCPII activity was exhibited in extracts of cabbage, lettuce and spinach. In contrast, this activity was significantly inhibited by extracts of orange juice (68.6% of no addition), tomatoes (66.9%) and green tea (35.6%). The activity was inhibited in a concentration-dependent manner by the extract of green tea. These results indicate that green tea may partially influence folate bioavailability through the inhibition of GCPII.

## Introduction

Folate is a water soluble B-group vitamin, which is an essential nutrient for human health. It is well known that folate deficiency causes neural tube defects and megaloblastic anemia[1, 2]. In addition, it has also been reported that the inadequate folate status was associated with cardiovascular disease and certain cancers in recent years[3, 4].

Pteroylpolyglutamate ($PteGlu_n$), the predominant form of dietary folates, is degraded to monoglutamyl form by zinc-dependent enzyme, glutamate carboxypeptidase II (GCPII), in the intestinal brush border membrane before absorption[5]. Although folates are found in a wide variety of foods, it has been reported that folate bioavailability differs among foods. The bioavailability of folates from various foods depends on a number of factors, including: the degradation of folates in the process of digestion, the inhibition of GCPII due to food components, the trapping of folates by food matrix and the presence of dietary constituents that may enhance folate stability during digestion[6, 7].

Green tea is the most widely consumed beverage in the world. It is one of the major sources of food folates. It has been reported that several foods inhibited human and porcine GCPII activities and that organic acids in orange juice and tomatoes inhibited the activity *in vitro*[8, 9]. However, it is not known whether green tea inhibits GCPII activity and influences folate bioavailability. In this study, we examined the influence of green tea on GCPII activity with the vesicle of porcine intestinal brush border membrane.

## Materials and Methods

Pteroyltriglutamate ($PteGlu_3$) was purchased from Dr. B. Schircks Laboratory (Jona, Switzerland). All other reagents were of the highest grade commercially available. Porcine intestine was obtained from the local slaughterhouse. Jejunal brush border membrane was isolated by a procedure described previously[8, 10]. All the food substances were obtained from a local grocery store. Green tea was prepared in the following manner: ten grams of dry green tea was added to 430 ml of distilled water at 90℃. After 1 min, this solution was filtrated and cooled to room temperature. All of the food was homogenized or mixed in 4 volumes of the extraction buffer (30 mM Tris-HCl buffer, pH 7.0, containing 100 mM NaCl and 0.1 mM zinc acetate) at 4℃ and then incubated in a boiling water bath for 30 min. The portions were then centrifuged at 10,000 x *g* for 20 min at 4℃. Supernatants were collected and adjusted to a pH of 7.0. These extracts were used to test in vitro effects on brush border GCPII activity.

GCPII activity was determined by previously described methods[9, 11]. In brief, the enzyme assay mixture

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(pH 7.0) contained porcine jejunal brush border membrane protein, zinc acetate and $PteGlu_3$ at 37℃ for 30 min. *In vitro* effects of various foods were evaluated by including extracts from them in the enzyme assay mixture. The hydrolysis of $PteGlu_3$ was determined by HPLC analysis using a $C_{18}$ column. The data were analyzed by one-way analysis of variance followed by Dunnett's multiple comparison test.

## Results and Discussion

Michaelis-Menten kinetics were observed for GCPII activity with $PteGlu_3$ as a substrate and the *K*m value was 0.81 ± 0.05 μM. Also, it was reported that the value was 1.0 ± 0.1 μM[12]. Therefore, $PteGlu_3$ concentration in the assay mixture was set at 1 μM when the effect of food extracts was examined. Table 1 shows that GCPII activity was almost unaffected by the addition of extracts of cabbage, lettuce and spinach. In contrasts, this activity was significantly inhibited by extracts of orange juice (68.6% of no addition), tomatoes (66.9%) and green tea (35.6%). These data show the activities when the extracts from the same amount of foods were added to the assay mixture. The concentration dependency of the inhibitory effect was also examined. Increasing concentrations of green tea and orange juice caused increasing inhibitory effects on the enzyme activity (Fig. 1).

**Table 1.** Effects of extracts from foods on GCPII activity in the porcine intestinal brush border membrane.

| Food | GCPII activity |
|---|---|
| | (pmol/min/mg protein) |
| Control (no addition) | 258.3 ± 16.6 |
| Cabbage | 255.6 ± 25.6 |
| Lettuce | 250.1 ± 4.0 |
| Spinach | 265.5 ± 10.7 |
| Tomatoes | 172.8 ± 8.9* |
| Orange juice | 177.1 ± 34.6* |
| Green tea | 91.8 ± 4.1* |

Values are means ± SD (n = 2-4). *Different from control, $p < 0.05$.

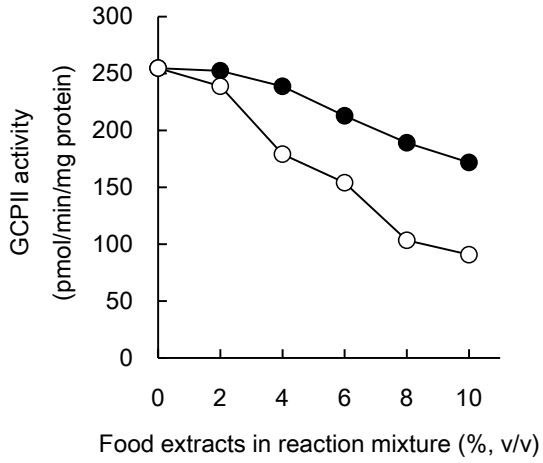


**Fig. 1** Effects of different concentration of food extracts on GCPII activity. Reaction mixtures contained varying concentrations of food extracts from green tea (open circle) and orange juice (closed circle).

Of the foods examined in this study, green tea in particular inhibited porcine brush border GCPII activity. Since GCPII is a zinc dependent enzyme, a 10-fold concentration of zinc acetate to the normal assay was added in the assay mixture. However, the inhibition by green tea was not recovered by the addition of zinc acetate (Fig. 2).

The bioavailability of dietary folates from a mixed diet is considered to be ≤50% of that of folic acid, but these estimations are different among studies. Incomplete bioavailability of natural food folates is considered to be caused by several factors. One of the factors is the inhibition of GCPII due to food components. For example, the bioavailability of pteroylheptaglutamate was reduced when added to orange juice in comparison with folic acid[13]. This resulted from the lowering of pH by the high load of orange juice. Some food components in beans[14] and orange juice[9] have been reported to act as GCPII inhibitors.

It was reported that organic acids, such as citric acid and malic acid, in orange juice and tomatoes inhibited the activity by a competitive mechanism[9]. In this study, orange juice and tomatoes also inhibited the GCPII activity. Interestingly enough, inhibition by green tea was higher than that by orange juice and tomatoes. Since the level of organic acids in green tea was much lower than orange juice and tomatoes, it was thought that the inhibitory mechanism was different. In addition, the inhibition by green tea was not mediated by the chelation of zinc

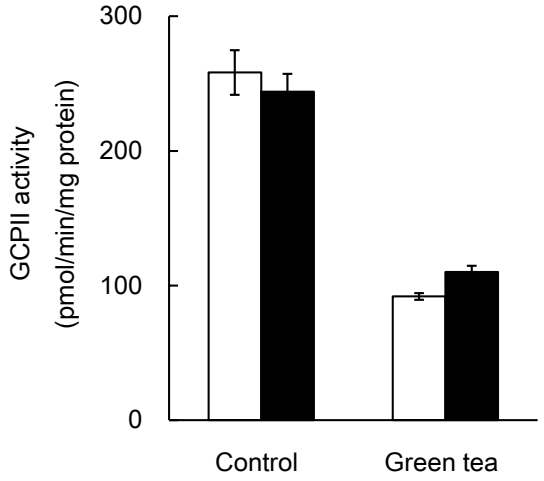


**Fig. 2** Effects of zinc ions on the inhibition of GCPII activity by green tea. Zinc acetate was included at 0.1 mM (open bar) or 1.0 mM (solid bar) in the reaction mixture. Values are means ± SD (n=3-4).

essential for GCPII activity. It is necessary to clarify the inhibitory compounds in green tea and the inhibitory mechanism.

There is growing interest in the relationship between tea and folate bioavailability. Inhibition of folic acid uptake by catechins and tea extract in Caco-2 cell was reported[15, 16]. Also, it was reported that green and black tea decreased bioavailability of folic acid in healthy adults[17]. In this study, we focused on the influence of green tea on the intestinal deconjugation by GCPII. It was also reported that the GCPII activity exceeds the needs for hydrolysis of food folates within the range of dietary intake[7]. Therefore, it is not yet known how important the inhibition of GCPII activity by green tea is in folate bioavailability.

It might be considered that the large intake of green tea influences the bioavailability of polyglutamyl folates in foods. However, this inhibition may be compensated by the high concentration of folates in green tea. In addition, it is obvious that green tea contributes substantially to human health as reported by many studies beyond this inhibition.

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