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Inhibition of pancreatic lipase by black tea theaflavins: Comparative enzymology and *in silico* modeling studies

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ABSTRACT

Few studies have examined the effect of black tea (*Camellia sinensis*) theaflavins on obesity-related targets. Pancreatic lipase (PL) plays a central role in fat metabolism and is a validated target for weight loss. We compared the inhibitory efficacy of individual theaflavins and explored the underlying mechanism. Theaflavin-3,3′-digallate (TFdiG), theaflavin-3′-gallate, theaflavin-3-gallate, and theaflavin inhibited PL with IC_{50} of 1.9, 4.2, 3.0, and >10 μmol/L. The presence and location of the galloyl ester moiety were essential for inhibitory potency. TFdiG exhibited mixed inhibition with respect to substrate concentration. *In silico* modeling showed that theaflavins bind to Asn263 and Asp206, which form a pocket adjacent to the active site, and galloyl-containing theaflavins are then predicted to perturb the protonation of His264. These data provide a putative mechanism to explain the anti-obesity effects of tea.

1. Introduction

In the United States, approximately 68% of adults are overweight or obese (Flegal, Carroll, Ogden, & Curtin, 2010; Ogden, Carroll, Kit, & Flegal, 2014). Although current data suggest that rates of obesity are stabilizing, obese and overweight individuals are at increased risk for a number of chronic diseases including diabetes, cardiovascular disease, and some forms of cancer (Noureddin & Rinella, 2015; Oda, 2012; Park, Morley, Kim, Clegg, & Scherer, 2014; Rani, Deep, Singh, Palle, & Yadav, 2016; Thomas et al., 2014).

Tea (*Camellia sinensis*, Theaceae) is the second most commonly consumed beverage in the world and approximately 80% of the tea consumed worldwide is in the form of black tea (Yang, Maliakal, & Meng, 2002). Theaflavins (Fig. 1) and thearubigins are characteristic polyphenols in black tea and result from the oxidation of tea leaves during the "fermentation" step of tea processing (Harbowy & Balentine, 1997). A typical cup of brewed black tea (2.5 g tea leaves in 250 mL) yields approximately 30% water-extractable solids (Yang et al., 2002). A survey of 32 black tea brands showed that the levels of theaflavin (TF), theaflavin-3-gallate (TF3G), theaflavin-3′-gallate (TF3′G), and theaflavin-3,3′-digallate (TFdiG) in the water-extractable solids were 0.1–5.4 mg/g, 0.2–2.1 mg/g, 0.1–0.8 mg/g, and 0.4–5.8 mg/g, respectively (Friedman et al., 2005). Epidemiological and laboratory animal model studies have demonstrated the potential efficacy of tea for weight management and obesity prevention (reviewed in (Grove & Lambert, 2010; Sae-tan, Grove, & Lambert, 2011)).

Although most laboratory studies have focused on the obesity preventive effects of green tea, there is growing evidence that black tea and theaflavins may also be useful for prevention of obesity (Yang, Zhang, Zhang, Huang, & Wang, 2016). Black tea polyphenols

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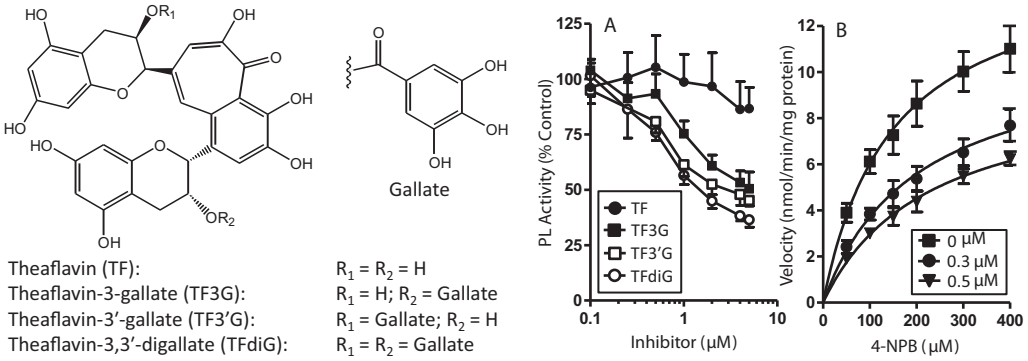

Fig. 1. Inhibition of pancreatic lipase by black tea polyphenols. (A) The dose-dependent inhibitory potency of theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate against pancreatic lipase *in vitro* was determined. The data was normalized to a vehicle-control. (B) Kinetic analysis was performed to determine the mode of enzyme inhibition of TFdiG. Incubations were carried out at 37 °C for 10 min using 4-NPB as the substrate and porcine pancreatic lipase as the enzyme source. Symbols represent the mean ± SEM of N = 3–6 independent experiments.

have been shown to significantly reduce serum triglyceride concentration in rats after co-administration of a liquid fat test meal and black tea extract compared to control treated rats (Kobayashi et al., 2009; Uchiyama, Taniguchi, Saka, Yoshida, & Yajima, 2011). Mice fed a high fat diet supplemented with 5% black tea polyphenol extract had significantly less body weight gain, parametrial adipose tissue mass, and liver lipid content compared to mice fed a high-fat diet (Uchiyama et al., 2011). These results suggest that black tea polyphenols may modulation dietary triglyceride digestion.

Pancreatic lipase (PL) is an enzyme secreted into the duodenum that plays a key role in the digestion and absorption of fats; in fact, PL may be responsible for cleaving 50–70% of dietary fats (Birari & Bhutani, 2007). Given the importance of PL for lipid digestion, it represents an attractive target for obesity prevention. Orlistat (tetrahydrolipstatin) is a Food and Drug Administration approved PL inhibitor marketed as a weight loss drug (Harrison, Fecht, Brunt, & Neuschwander-Tetri, 2009).

We have previously reported that (−)-epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, dose-dependently inhibits PL *in vitro* (IC_{50} = 7.5 μmol/L); this inhibition was non-competitive with respect to substrate concentration (Grove, Sae-tan, Kennett, & Lambert, 2012). By contrast, (−)-epigallocatechin, which has no galloyl ester, was ineffective. Wang et al., recently reported similar results (Wang, Sun, Dong, Liu, & Liu, 2014). An *in silico* model for the interaction between EGCG and PL was proposed by the same group, but the authors used a PL dimer as a protein structure (Wu et al., 2013). Although the crystal structure of PL was solved using the dimer, this is not the enzymatically active structure of PL, and conclusions regarding molecular interactions based on this structure are spurious. In addition, the authors used an incorrect structure for EGCG.

Less is known about the effects of the theaflavins on PL. Nakai et al. have reported that oolong tea polyphenols, including some theaflavin isomers, inhibit PL *in vitro* (Nakai et al., 2005). IC_{50} values ranged from 0.068 μmol/L (oolongtheanin 3′-*O*-gallate) to >20 μmol/L ((−)-epigallocatechin). In each of these studies, polyphenols containing a galloyl ester had significantly greater inhibitory potency than non-galloyl containing molecules. The authors provided no information vis-à-vis the enzymatic mechanism of inhibition.

It has been posited that the requirement of the galloyl ester indicates that the ester moiety competes with the esters in triglycerides resulting in inhibition of lipase-mediated cleavage. Although interesting, this mechanism fails to account for 1) the lack of formation of the de-esterified polyphenol in the reaction mixture and 2) the non-competitive mode of inhibition reported for EGCG.

The objectives of the present study were to compare the PL inhibitory activity of purified black tea theaflavins, to determine the inhibitory kinetics of the most potent theaflavin, and to develop an *in silico* model to better understand the inhibitory activity of the theaflavins.

2. Materials and methods

2.1. Chemicals

Lipase (type II, from porcine pancreas, specific activity = 400 U/mg), 4-nitrophenyl butyrate (4-NPB), and orlistat were purchased from Sigma Chemical Company (St. Louis, MO). Theaflavin (TF, 98% pure), theaflavin-3-gallate (TF3G, 98% pure), theaflavin-3′-gallate (TF3′G, 98% pure), and theaflavin-3,3′-digallate (TFdiG, 98% pure) were purchased from Quality Phytochemicals LLC (Edison, NJ). All other chemicals were of the highest grade commercially available.

2.2. Pancreatic lipase activity

PL was suspended in water (10 mg/mL) and incubated at 37 °C for 5 min. The solution was centrifuged for 5 min at 664*g* and the supernatant was then used as the enzyme source for subsequent experiments. For each experiment, the PL supernatant was diluted 1: buffer solution (20 mmol/L Tris-HCl, 1.3 mmol/L $CaCl_2$, 150 mmol/L NaCl, pH = 8.0) and combined with the inhibitor of interest. The reaction was then started by the addition of 4-NPB (226 μmol/L final concentration). After incubation at 37 °C for 10 min the absorbance was measured spectrophotometrically at 400 nm. Based on previous work in our laboratory, the reaction rate is linear over the time-frame of the experiment. Absorbance values were normalized to the vehicle control and dose–response curves were prepared.

The mode of enzyme inhibition was determined in a manner similar to that described above with the modification that the inhibitor concentration was held constant and 4-NPB concentration (0–500 μM) was varied. The maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) were determined by fitting the initial velocity as a function of concentration of 4-NPB.

2.3. In silico modeling

Initial atomic coordinates for the inhibitors were built using the online PRODRG server (Schuttelkopf & van Aalten, 2004). The inhibitors thus generated were manually moved and rotated into the binding pocket adjacent to the active site pocket of the PL