A decaffeinated green coffee bean extract (DGCBE) inhibited porcine pancreas lipase (PPL) activity with an IC₅₀ value of 1.98 mg/mL. Six different chlorogenic acids in DGCBE contributed to this PPL inhibition, accounting for 91.8% of the inhibitory activity. DGCBE increased the droplet size and decreased the specific surface area of an olive oil emulsion.

**Key words:** coffee; decaffeinated green coffee bean extract; chlorogenic acid; emulsion; lipase inhibitor

Coffee is one of the most popular beverages in the world and is a major source of polyphenols, particularly chlorogenic acids (CGAs). CGAs in green coffee beans consist of three main classes: caffeoylquinic acids (CQAs) with three isomers (3-, 4-, and 5-CQA), dicaffeoylquinic acids (diCQAs) with three isomers (3,4-, 3,5-, and 4,5-diCQA), and feruloylquinic acids (FQAs) with three isomers (3-, 4-, and 5-FQA). Green coffee beans are rich in CGAs, their contents being 3.5–7.5% (w/w of dry matter) for *Coffea arabica* and 7.0–14.0% (w/w of dry matter) for *Coffea canephora*. These nine CGAs account for 80% of the total CGAs in green coffee beans.

Porcine pancreas lipase (EC 3.1.1.3, hereafter abbreviated as PPL) is a water-soluble enzyme that catalyzes the hydrolysis of the ester bonds of triacylglycerols to form monoacylglycerol and two fatty acids at the interface between an insoluble substrate and water. PPL is composed of a single polypeptide chain of 449 amino acid residues (50 kDa) connected to a glycan chain (2 kDa) and has 86% homology with human pancreas lipase. Pancreas lipase is a key enzyme for fat digestion in the intestines.

Lipase inhibitors appear to be effective for preventing obesity and for its therapy by controlling the absorption of fat in the diet, as this fat is an energy source with a high caloric content. Lipase inhibitors are also used to prevent such undesirable changes in foods as offensive flavors caused by lipases.

We investigated the inhibitory effects of a decaffeinated green coffee bean extract (DGCBE) on PPL activity. DGCBE was obtained from Ominedo Pharmaceutical Industries (Nara, Japan). The activity of PPL in the absence and presence of DGCBE was determined according to a previously reported method with some modifications. A total of 80 mg of triolein, 10 mg of lecithin, and 5 mg of sodium taurocholic acid were added to 9 mL of a 0.1 M 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) buffer (pH 7.0). An emulsion was prepared by ultrasonication of this mixture. DGCBE samples (40 μL) of different concentrations or 40 μL of the 0.1 M HEPES buffer (control) were mixed with 40 μL of the emulsion and incubated at 37 °C for 30 min. A 20-μL amount of PPL (15,000 U/mL) was added to the mixture which was then incubated at 37 °C for 30 min. The reaction was terminated by boiling for 3 min. Free fatty acids in the mixture were determined by using a NEFA C-test kit for free fatty acids (Wako Pure Chemical, Osaka, Japan). The IC₅₀ value was determined from a least-squares regression line after plotting the natural logarithms of the sample concentrations against the remaining PPL activity. The inhibitory effects of DGCBE increased with increasing inhibitor concentration, with an IC₅₀ value of 1.98 mg/mL (Table 1).

Green coffee beans are rich in CGAs that have such biological effects as antioxidative activity, matrix metalloproteinase inhibition, tyrosinase inhibitory activity, and α-amylase inhibitory activity. We evaluated the inhibitory effects against PPL of 3-CQA, 4-CQA, 3-FQA, 4-FQA, 5-FQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA, each of which was purified from DGCBE as described in our previous paper, and of 5-CQA which was purchased (Nacalai Tesque, Kyoto, Japan). The inhibitory effects of these nine different CGAs against PPL increased with increasing CGA concentration (Fig. 1); their IC₅₀ values are shown in Table 1. The inhibitory activities of such caffeic acid derivatives as CQAs and diCQAs were higher than those of such ferulic acid derivatives as FQAs, suggesting that the two neighboring hydroxyls on the catechol ring were effective for this inhibitory activity.

The contents of eight of the CGAs in DGCBE have been previously reported. The contents of 3-CQA, 4-CQA, 5-CQA, 4-FQA, 5-FQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA in DGCBE were 5.12, 7.03, 14.8, 1.29, 3.84, 2.10, 1.59, and 3.04 g/100 g, respectively. The relative contributions of six of these CGAs to the IC₅₀ value...
of DGCBE were calculated as follows:\textsuperscript{8,10} Relative contribution (%) = $\frac{IC_{50,\text{DGCBE}} \times C_{\text{CGA}}}{IC_{50,\text{CGA}}}$ where $IC_{50,\text{DGCBE}}$ is the $IC_{50}$ value of DGCBE, $C_{\text{CGA}}$ is the amount (%) of each chlorogenic acid in DGCBE, and $IC_{50,\text{CGA}}$ is the $IC_{50}$ value of each chlorogenic acid. The theoretical relative contributions to PPL inhibition by 3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA against DGCBE were respectively calculated as 9.3%, 11.1%, 26.2%, 13.0%, 8.1%, and 24.1% (Table 1). The total relative contribution of these six CGAs to the inhibitory activity of DGCBE was 91.8% (Table 1). These six CGAs were thus major contributors to the inhibition of PPL activity by DGCBE. In particular, 5-CQA and 4,5-diCQA mainly contributed to the inhibitory activity of DGCBE against PPL (Table 1).

PPL inhibition is mainly classified into two types.\textsuperscript{11–13} One type of inhibition is due to direct interaction between the inhibitor and enzyme, and the other is due to the inhibitor affecting the properties of the fat emulsion such as the droplet size and specific surface area, because the lipase activity is sensitive to the size and specific surface area of the fat emulsion.\textsuperscript{14–17} It has been reported that a green tea extract and catechins had effects on the physicochemical properties of an olive oil emulsion by increasing its droplet size and decreasing its specific surface area.\textsuperscript{14}

We evaluated the effects of DGCBE on the droplet size and the specific surface area of an olive oil emulsion by using a previously reported method.\textsuperscript{14} An olive oil emulsion was prepared by mixing taurocholic acid (8.0 mM final concentration), olive oil (1% v/v), phosphatidylcholine (2.3 mM), NaCl (150 mM), and CaCl$_2$ (10 mM) in a 2 mM Tris–HCl buffer (pH 7.5). A sample (3 μL) was added to 20 mL of this mixture, and the pH value was readjusted to 7.5 with NaHCO$_3$. The mixture was incubated at 37°C while constantly shaking (100 stokes/min) for 3 h. The droplet size and specific surface area of the olive oil emulsion were each measured by an LA-920 laser scattering particle size distribution analyzer (Horiba, Kyoto, Japan). We used distilled water as a negative control, and a catechin mixture from green tea (Wako) as a positive control. Compared with the negative control emulsion, the droplet size increased and the specific surface area decreased for the emulsion with the catechin mixture (Table 2). This result was in agreement with a previous report.\textsuperscript{14} The droplet size and specific surface area of the emulsion with DGCBE also respectively increased and decreased (Table 2). This phenomenon indicates that at least one of the inhibitory mechanisms of DGCBE was reducing the surface area of the lipid emulsion because the lipase activity is governed by the area of the oil-water interface.\textsuperscript{14–17}

It has been reported that a competitive inhibition model accounted for the inhibitory effects of both chlorogenic acid (5-CQA) and caffeic acid against the rice bran lipase-catalyzed hydrolysis of triacetin which is a water-soluble substrate.\textsuperscript{18} Their respective inhibition constants ($K_i$) were 1.8 μM and 1.5 μM.\textsuperscript{19} The PPL

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**Table 1. IC$_{50}$ for DGCBE and Nine Kinds of CGAs and the Contribution of Six Kinds of CGAs**

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (mg/mL)</th>
<th>IC$_{50}$ (μM)</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGCBE</td>
<td>1.98 ± 0.21</td>
<td>1.88 ± 0.19</td>
<td>—</td>
</tr>
<tr>
<td>3-CQA</td>
<td>1.09 ± 0.08</td>
<td>3.09 ± 0.25</td>
<td>9.3</td>
</tr>
<tr>
<td>4-CQA</td>
<td>1.25 ± 0.10</td>
<td>3.53 ± 0.26</td>
<td>11.1</td>
</tr>
<tr>
<td>5-CQA</td>
<td>1.12 ± 0.04</td>
<td>3.15 ± 0.12</td>
<td>26.2</td>
</tr>
<tr>
<td>3-FQA</td>
<td>&gt;2.95</td>
<td>&gt;8.00</td>
<td>—</td>
</tr>
<tr>
<td>4-FQA</td>
<td>&gt;2.95</td>
<td>&gt;8.00</td>
<td>—</td>
</tr>
<tr>
<td>5-FQA</td>
<td>&gt;2.95</td>
<td>&gt;8.00</td>
<td>—</td>
</tr>
<tr>
<td>3,4-diCQA</td>
<td>0.32 ± 0.02</td>
<td>0.62 ± 0.04</td>
<td>13.0</td>
</tr>
<tr>
<td>3,5-diCQA</td>
<td>0.39 ± 0.04</td>
<td>0.75 ± 0.08</td>
<td>8.1</td>
</tr>
<tr>
<td>4,5-diCQA</td>
<td>0.25 ± 0.03</td>
<td>0.48 ± 0.07</td>
<td>24.1</td>
</tr>
</tbody>
</table>

*Values are the mean ± SD (n = 5).*

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**Table 2. Droplet Size and Specific Surface Area of Olive Oil Emulsions with and without a Sample**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. (mg/mL)</th>
<th>Droplet size (μm)</th>
<th>Specific surface area (m$^2$/g oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>—</td>
<td>5.2 ± 0.1</td>
<td>1.6 ± 0.0</td>
</tr>
<tr>
<td>DGCBE</td>
<td>1.0</td>
<td>14.9 ± 2.9</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>DGCBE</td>
<td>5.0</td>
<td>17.2 ± 3.5</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Catechin mixture</td>
<td>1.0</td>
<td>123.0 ± 4.9</td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

*Values are the mean ± SD (n = 3).*

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**Fig. 1.** Inhibitory Effects of Nine Different Chlorogenic Acids against the PPL-Catalyzed Hydrolysis of Triolein in an Emulsion.

The inhibition assay was performed at pH 7.0 and 37°C. A: 5-CQA (unfilled circles), 4-CQA (unfilled triangles), and 3-CQA (unfilled squares). B: 5-FQA (filled circles), 4-FQA (filled triangles), 3-FQA (filled squares), 3,4-diCQA (unfilled diamonds), 3,5-diCQA (filled diamonds), and 4,5-diCQA (inverted unfilled triangles). Each point represents an average of 5 measurements, and error bars indicate ± SD.
inhibition model for DGCBE is quite complex and requires further study. We are currently investigating the PPL inhibitory mechanism of DGCBE. The inhibition mechanisms of some inhibitors from foods have been reported.\textsuperscript{12,13,19} It has been suggested that proteins from soy bean seeds inhibited PPL, and that the cause was not by the direct interaction between PPL and the inhibitor, but between the inhibitor and substrate emulsion.\textsuperscript{12,13} Goncalves \textit{et al.} have reported that procyanidins from grape seeds inhibited PPL and that this inhibition was caused by the interaction between the inhibitor and the enzyme at a very high stoichiometric ratio before PPL inhibition.\textsuperscript{19}

The IC\textsubscript{50} value of 5-CQA for the PPL-catalyzed hydrolysis of triolein was 3.15 mM in this study. Raghavendra \textit{et al.} have reported that the \textit{K}_i value of 5-CQA for the rice bran lipase-catalyzed hydrolysis of triacetin was 1.8 \textmu M.\textsuperscript{11} There seem to be two factors concerning the differences in these results, one possibly being due to the different substrates used for the experiments. We used triolein as a water-insoluble substrate, but Raghavendra \textit{et al.} used triacetin as a water-soluble substrate. Kobayashi \textit{et al.} have reported that the inhibitory effects of catechins and theaflavins against the PPL-catalyzed hydrolysis of triolein, which is a water-insoluble substrate in an emulsion, were much weaker than those against the PPL-catalyzed hydrolysis of 4-methylumbelliferone which is a water-soluble substrate.\textsuperscript{51} The other factor concerning the differences in these results is the kind of lipase used; we used PPL, but Raghavendra \textit{et al.} used rice bran lipase. It has been reported that globulins material from garden radish seeds had markedly high inhibitory activity against \textit{Rhizopus delemar} lipase, but not much against PPL.\textsuperscript{11} Further investigation is needed to elucidate the activities of various lipases on different substrates in the presence and absence of 5-CQA.

In conclusion, we have shown that DGCBE had an inhibitory effect against the PPL catalysis of triolein in an emulsion and that six CGAs contributed to this inhibitory activity. At least one of the inhibitory mechanisms of DGCBE was reducing the surface area of a lipid emulsion. These observations provide significant clues for the development of lipase inhibitors that may be useful for preventing obesity and for its therapy.

References