Preliminary Communication

Inhibition of Adrenalin-induced Lipolysis by Ginseng Polypeptide and Its Modified Peptides

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The anti-lipolysis by ginseng polypeptide and its modified peptides was examined using porcine adipose cells. Ginseng polypeptides modified by amino acid substitution or proteolyzed reduced or lost the inhibiting activity of adrenalin-induced lipolysis. Correlation between the anti-lipolytic activity of ginseng polypeptide and its Mg²⁺- and ribose-binding activities is discussed.

Key words: adrenalin; anti-lipolysis; bovine intramuscular preadipocyte cell; porcine adipose cell; Panax ginseng

Roots of ginseng (Panax ginseng C. A. Meyer), a traditional medicinal plant, contain numerous regulatory chemicals and peptides. One of these chemicals, a ginseng polypeptide (GPP) which is composed of 14 amino acids and found in water extracts of ginseng roots, has been reported to be an inhibitor of the adrenalin-induced lipolysis in an isolated rat fat cell assay system. We previously found evidences of metal- and ribose-binding activities of GPP using affinity capillary electrophoresis and surface plasmon resonance.

In this communication, we report the anti-lipolytic activity of GPP and modified GPPs using isolated porcine adipose cells and a bovine intramuscular preadipocyte (BIP) cell line. Correlation between this activity and Mg²⁺- and ribose-binding activities is discussed.

The synthesis and the purification of GPP, modified GPPs, and parvalbumin-like peptide (PV) were previously described (Fig. 1). Proteolysis of GPP was done with V8 proteinase (Wako Pure Chem., Tokyo, Japan) and proteinase K (Wako) with overnight incubation.

The porcine abdominal adipose tissue was obtained at a local slaughterhouse. Adipose cells were isolated from 10 g of adipose tissue by digestion with 4 mg of collagenase in 10 ml of Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing 2% bovine serum albumin (BSA) for 1 hr at 37°C. Lipolysis was immediately allowed to proceed in the cell suspension in KRB containing 2% BSA. Lipolysis in eight-day-old differentiated BIP cells was allowed to proceed in 6-well plates in serum-free Dulbecco’s modified Eagle medium containing 2% BSA.

The peptides and 100 mg/1 D,L-adrenalin (Wako) were added to the incubation medium to start lipolysis in cells for 1 hr at 37°C. The incubations were ended by placing the containers on ice. The released glycerol in incubation medium were measured using an F-kit glycerol (Boehringer Mannheim, Germany).

Inhibition of the adrenalin-induced lipolysis by GPP and proteolyzed GPPs was monitored by the release of glycerol from adipose cells (Fig. 2 A). Neither of the proteolyzed GPPs had the anti-lipolytic activity. An acidic PV, which was selected as having similar characteristics in size and acidity to GPP was not effective in inhibition of the lipolysis. Thus, the lipolysis was not merely inhibited by the acidic nature of the peptides.

The anti-lipolytic activity of GPP and modified GPPs was examined using the porcine adipose cells (Fig. 2 B). Peptide-1 and peptide-2 reduced the anti-lipolytic activity compared to that of GPP, indicating that the substitution of acidic residues between E and D could affect the activity. Though G11DA inhibited the lipolysis, it released more glycerol than GPP. The peptides that neutralized one acidic amino acid, E1Q, E4Q, and D7N, yielded glycerol at almost the same level as the control, which indicates the N-terminal region of GPP was involved in the activity.

The anti-lipolytic activity of GPP and modified GPPs was confirmed using the differentiated BIP cells (Data

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Abbreviations: BIP, bovine intramuscular preadipocyte; BSA, bovine serum albumin; dA, D-alanine; GPP, ginseng polypeptide; KRB, Krebs-Ringer bicarbonate; PV, parvalbumin-like peptide.
Fig. 2. Inhibition of Adrenaline-induced Lipolysis by GPP and Proteolyzed GPPs (A) and by GPP and Modified GPPs (B) in Porcine Adipose Cells.

Concentration of peptides was 1.0 ng/ml. Data are expressed as the mean ± SD of triplicate assays. Data were analysed by one-way analysis of variance (ANOVA). Values not sharing a common letter are significantly different at P < 0.05 by Duncan’s multiple range test.

Table. Inhibition of Lipolysis and Mg$^{2+}$- and Ribose-binding Activities of GPP and Its Modified Peptides.

<table>
<thead>
<tr>
<th>Inhibition of lipolysis</th>
<th>Binding activity</th>
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<tbody>
<tr>
<td>Porcine</td>
<td>BIP</td>
</tr>
<tr>
<td>GPP</td>
<td>+ + +</td>
</tr>
<tr>
<td>Peptide-1 (#1)</td>
<td>±</td>
</tr>
<tr>
<td>Peptide-2 (#2)</td>
<td>±</td>
</tr>
<tr>
<td>E1Q</td>
<td>–</td>
</tr>
<tr>
<td>E4Q</td>
<td>–</td>
</tr>
<tr>
<td>D7N</td>
<td>±</td>
</tr>
<tr>
<td>G11dA</td>
<td>+</td>
</tr>
<tr>
<td>V8 proteolyzed</td>
<td>–</td>
</tr>
<tr>
<td>ProK proteolyzed</td>
<td>–</td>
</tr>
</tbody>
</table>

n.t., not tested. + + +, strongly positive; +, positive; –, negative. V8, V8 proteinase; ProK, proteinase K.

Fig. 3. Amino Acid Sequence Similarity between GPP and Neuropeptide Y and Peptide YY.

not shown). The BIP cells gave similar results to those in the porcine lipolysis experiments (Table).

As reported previously, we found that GPP bound Mg$^{2+}$ and ribose,4,11 the results of which were summarized in the Table. The binding regions of Mg$^{2+}$ and ribose in GPP could be speculated on based on Table and Fig. 1. The loss of anti-lipolysis activity in E1Q and E4Q was in parallel with the loss of Mg$^{2+}$-binding activity. This suggests that the N-terminal region is involved in Mg$^{2+}$-binding through which GPP contributes to the anti-lipolysis. In peptide-1, where E9 was replaced, ribose-binding activity was lost. This marked discrepancy suggests that the C-terminal region is important for ribose binding.

Takig these results into consideration, the inhibition mechanism in the adrenalin-induced lipolysis by GPP could be hypothesized as follows: First, GPP might inhibit lipolysis by chelating extracellular Ca$^{2+}$.4 The entry of Ca$^{2+}$, which was observed in the initial stage of lipolysis,12 was regulated by binding adrenalin to the α-adrenergic receptor. Second, GPP might bind intracellular Mg$^{2+}$ to inhibit the signal transduction. β-Adrenergic receptors transduce the signal into cells through G-protein, adenylate cyclase, and cAMP,13 which require Mg$^{2+}$ for their stabilities. Third, inhibition of lipolysis comes from the ribose-binding activity of GPP. GPP might inhibit lipolysis by binding ribose present in ATP and cAMP. Finally, GPP might bind polysaccharides on the adrenergic receptors.12,13 The binding site of adrenalin was reported to be close to the glycylsation site on the adrenergic receptor. In another experiment, GPP was confirmed to bind glucose as well as ribose (Data not shown). Together with the facts that the sequence of GPP is highly similar to motif-2 sequence in human adenosine kinase and microbial pentose and hexose kinases,15 it might be possible that GPP binds to the polysaccharides on the adrenalin-receptors. Among these working hypotheses, we speculated that ribose-binding activities played the most important role in the inhibition of lipolysis in GPP because the β$\text{a}$-adrenergic receptor, which transduces signals by cAMP is mainly expressed in adipose cells. More physiological studies are needed to know the actual function of GPP that contributes to the inhibition of adrenaline-induced lipolysis.

As far as we know, there were two peptides other than insulin which inhibit the adrenalin-induced lipolysis, neuropeptide Y and peptide YY in rat.14,15 We found sequence similarity between GPP and these two peptides, especially in some amino acids which are involved in the anti-lipolytic activity (Fig. 3). Though binding activities of these peptides to Mg$^{2+}$ and ribose are not known, it is interesting to compare the inhibitory mechanism be-
between GPP and these peptides. As a unique inhibitor of adrenalin, GPP can be expected not only as a tool for the understanding of adrenalin-mediated biological responses but also as an anti-lipolysis drug in the future.

Acknowledgment
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References
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