THE SUBSTRATE SPECIFICITY OF GLYCEROL ESTER HYDROLASE FROM PIG AORTA AND SERUM

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The activity of glycerol ester hydrolase (GEH) from aorta wall, at optimum pH and triacylglycerol substrate concentration (optimal for each substrate) decreased in the following order: \( C_{18:1} \geq C_{18:2} \geq C_{18:0} \geq C_{18:3} \geq C_{16:0} \). At optimum pH and the same substrate concentration (1 mM), the activity of GEH from aorta wall decreased in a slightly different order: \( C_{18:1} \geq C_{18:2} \geq C_{18:3} \geq C_{16:0} \geq C_{18:0} \) and that of the enzyme from serum in the order: \( C_{18:1} = C_{18:3} > C_{18:2} \geq C_{16:0} \geq C_{18:0} \). These differences in substrate affinity of GEH may influence the metabolism and accumulation of acylalcohols and alcohols in arterial wall and serum.

Triacylglycerols (TAG) are the main components of blood plasma lipoproteins; however, their role in pathogenesis of arteriosclerosis has not been fully elucidated. TAG depending on their origin, consist of a diversified mixture of compounds differing both in chain length and degree of saturation of fatty acids. Since these compounds are quantitatively, the most important lipid component of food, their decomposition and resorption play a significant role.

Triacylglycerols are decomposed by glycerol ester hydrolase (GEH, EC 3.1.1.3). Also they can be transformed by triacylglycerol:cholesterol transacylase (EC 2.3.1). The enzymes catalysing hydrolysis and transacylation have been found both in aorta wall and in serum [1, 2, 3]. Changes in the activity of these enzymes may affect the metabolism of TAG, and that of cholesterol esters and phospholipids. Recent studies demonstrated differences in the activity of a number of lipolytic enzymes of aorta wall when tested with substrates differing in chain length and degree of saturation of fatty acid [4, 5, 6].

The aim of the present work was to study the effect of triacylglycerol concentration and fatty acid saturation on the activity of GEH from arterial wall and blood serum.

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MATERIALS AND METHODS

Aqueous extracts from acetone-butanol dried human serum and pig main arteries were obtained as described previously [4]. Protein was determined according to Lowry et al. [7]. Fresh alcohol-water solutions of triacylglycerols [8] were prepared for each experiment. The reaction mixture contained in a total volume of 7 ml: reduced glutathione (0.1 mmol/l), calcium chloride (0.5 mmol/l), tris(hydroxymethyl)aminomethane buffer (20 mmol/l) the enzymatic extract (0.2 g protein/l) and alternatively triacylglycerols: I, tripalmitoylglycerol; II, tristearoylglycerol; III, trioleylglycerol; IV, trilinoeoylglycerol; V, trilinolenoylglycerol (at concentration equal to 1 mM or optimal for each substrate; see Table 1).

The enzyme activity was determined by measuring the increase in the amount of fatty acids liberated after 20 min incubation at 30°C. Fatty acids were extracted according to Dole [9] using 2.5 ml of the extraction mixture per 1 ml sample, and were quantified spectrophotometrically according to Mosinger [10]. The enzyme activity was expressed in μmol × min⁻¹ per 1 mg protein, and were evaluated statistically.

RESULTS

The effect of concentration of various triacylglycerol substrates on GEH activity from arterial wall is presented in Fig. 1. The maximum enzyme activity was observed at the following substrate concentrations: 1.0 mM for trioleyl-, trilinoeoyl- and trilinolenoylglycerol, 1.25 mM for tristearoylglycerol, and 2.0 mM for plamitoylglycerol. Higher substrate concentrations were inhibitory.

As shown in Fig. 2 two peaks of GEH activity from aorta wall were observed for trilinoeoylglycerol at pH 7.3 and 8.6, (at higher pH the activity was about 1/3 lower) and for trilinolenoylglycerol at pH 7.3 and 8.3, with the activity at higher pH lower by only 10%. A single peak of the activity at pH 8.0 was found for tripalmitoylglycerol and trioleylglycerol and at pH 8.3 for tristearoylglycerol.

The maximum GEH activity with the above substrates under optimal conditions (pH and concentration) is presented in Table 1. The differences in GEH activity with TAG substrate containing saturated fatty acids (C₁₆:0, C₁₈:0) and poly-unsaturated fatty acids were insignificant except that the enzyme activity with trioleylglycerol was significantly higher than that with saturated tripalmitoylglycerol and poly-unsaturated trilinolenoylglycerol.

The comparative data (Table 2), on the activities of the enzyme from arterial wall and blood serum at the same substrate 1 mM concentration and at optimum pH showed higher activity of GEH from arterial wall towards triacylglycerol substrates containing 18 carbon atoms than with tripalmitoylglycerol. Significant differences with the serum enzyme concerned
Fig. 1. The effect of substrate concentration on the activity of glycerol ester hydrolase from arterial wall. I, Tripalmitoylglycerol; II, tristearoylglycerol; III, trioleylglycerol; IV, trilinoleylglycerol; V, trilinolenoylglycerol.

Fig. 2. The effect of pH of the incubation mixture on enzymatic hydrolysis of substrates (designations as in Fig. 1)
Table 1

The activity of glycerol ester hydrolase from arterial wall with triacylglycerols under optimum conditions (of concentration and pH) for each substrate

The activity is expressed in nanomoles of fatty acid released per minute per 1 mg protein; the results are mean values from the number of experiments indicated ± S.D. Statistical analysis based on Student's t test.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Substrate conc. (mM)</th>
<th>pH</th>
<th>No. of expts.</th>
<th>Activity (nmol × min⁻¹ × mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tripalmitoylgllycerol</td>
<td>2.0</td>
<td>8.0</td>
<td>10</td>
<td>8.5 ± 1.6</td>
</tr>
<tr>
<td>Tristearoylglycerol</td>
<td>1.25</td>
<td>8.3</td>
<td>21</td>
<td>9.1 ± 1.8</td>
</tr>
<tr>
<td>Trioleylglycerol</td>
<td>1.0</td>
<td>8.0</td>
<td>26</td>
<td>10.5 ± 1.5b</td>
</tr>
<tr>
<td>Trilinoleylglycerol</td>
<td>1.0</td>
<td>7.3</td>
<td>11</td>
<td>9.5 ± 1.4</td>
</tr>
<tr>
<td>Trilinolenoylglycerol</td>
<td>1.0</td>
<td>7.3</td>
<td>10</td>
<td>8.5 ± 1.5</td>
</tr>
</tbody>
</table>

Means were compared with that for tripalmitoylgllycerol and statistically different values at P ≤ 0.05 were marked by letter b.

Table 2

The activity of glycerol ester hydrolase from arterial wall and blood serum with triacylglycerols at substrate concentration of 1 mM and optimum pH for each substrate

<table>
<thead>
<tr>
<th>Substrate</th>
<th>no. of expts.</th>
<th>Arterial wall activity (nmol × min⁻¹ × mg⁻¹)</th>
<th>Serum activity (nmol × min⁻¹ × mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tripalmitoylgllycerol  ( C_{16:0} )</td>
<td>9</td>
<td>5.3 ± 0.9</td>
<td>7.7 ± 0.9c</td>
</tr>
<tr>
<td>Tristearoylglycerol    ( C_{18:0} )</td>
<td>8</td>
<td>8.3 ± 1.3</td>
<td>6.8 ± 0.8c</td>
</tr>
<tr>
<td>Trioleylglycerol ( C_{18:1} )</td>
<td>26</td>
<td>10.5 ± 1.5</td>
<td>10.7 ± 1.5b</td>
</tr>
<tr>
<td>Trilinoleylglycerol    ( C_{18:2} )</td>
<td>11</td>
<td>9.5 ± 1.4</td>
<td>9.0 ± 1.2</td>
</tr>
<tr>
<td>Trilinolenoylglycerol  ( C_{18:3} )</td>
<td>10</td>
<td>8.5 ± 1.5</td>
<td>10.6 ± 0.7b, c</td>
</tr>
</tbody>
</table>

Means for \( C_{18:0} - C_{18:3} \) were compared with that for \( C_{16:0} \) within and between groups I and II and significantly different values at P ≤ 0.05 were marked by b and c, respectively.

trioleylglycerol and trilinolenoylglycerol. The enzyme from arterial wall and that from serum showed differences in the activities with tripalmitoyl-, tristearoyl- and trilinolenoylglycerol.

DISCUSSION

The results obtained indicate that GEH activity depends on the kind of fatty acid in an acylglycerol substrate. Hydrolysis of triacylglycerols is a complex reaction in which the water-soluble enzyme reacts with the insoluble substrate. The enzyme-substrate interactions take place at the water-lipid interphase, and therefore the enzyme activity could be affected by the physical state: size of the substrate molecules, concentration and external charge of the
substrate [11]. The elaborated conditions made, however, possible measuring of hydrolytic activity with various triacylglycerols. Our preliminary data on substrate specificity of GEH from arterial wall and serum, at the same substrate concentration and at optimum pH, point to a preference of trioleylglycerol as a substrate (Table 2).

This was confirmed on testing the enzyme under optimum conditions (Table 1). Also at the same triacylglycerol concentration used it was possible to relate the enzyme activity with molecular weight and saturation of the substrate. This relation might be of significance for the metabolism and accumulation of acylcholesterols [12]. Oleic acid is a preferable substrate of acyl-CoA:cholesterol acyltransferase (ACAT) whereas oleylcholesterol is hydrolysed at the slowest rate among all acylcholesterols [5] and forms the main component of sclerotic deposits.

Studies on formation and degradation of the oleic acid complex with the enzyme, protein isolated from arterial wall, [13] also pointed to a specific role of this acid.

The enzyme studied resembles in many respects cholesterol ester hydrolase of arterial wall [5]. GEH, similarly as the latter enzyme, is inhibited by the excess substrate and shows double pH maxima. This could be due to the presence of isoenzymes, or to changes in conformation of poly-unsaturated substrates.

Although the results obtained do not permit an unequivocal determination of substrate affinities, they stress out the significance of hydrolytic process in removal of triacylglycerols and, indirectly, in the synthesis of acylcholesterols by the enzyme systems acting on the degradation products of acylglycerols.

REFERENCES