Hypocholesterolemic Effect of Phototrophic Bacterial Cells in Rats

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Summary The effect of diets containing the cells of a phototrophic bacteria (PTB), Rhodopseudomonas capsulate, on lipid metabolism was examined in the serum and liver of rats. Three groups of rats, 5 animals per group, were fed either a diet containing 0.2 or 2.0% PTB cells or a casein-based control diet. Each diet contained 1% cholesterol (CHOL). While serum glucose levels were not significantly different between the control and the PTB groups, total CHOL and triacylglycerol (TG) in the serum were significantly lower in the PTB groups than in the control group (p<0.01). The ratio of serum HDL-CHOL to total serum CHOL was significantly higher in the PTB groups than in the control group (p<0.01). The 2.0% PTB group had lower hepatic TG (p<0.05) but higher hepatic CHOL (p<0.05) than did the control group. These results indicate that PTB cells contain a factor or factors which affect hepatic metabolism or secretion of CHOL.

Key Words phototrophic bacteria (PTB), casein, serum cholesterol, hypocholesterolemic effect, HDL-cholesterol

Phototrophic bacteria (PTB) grow in mixed cultures with aerobic, heterotrophic, and nitrogen-fixing bacteria, giving biomass of 1 to 2 g/liter. PTB such as Rhodopseudomonas capsulate have been successfully employed in the treatment of industrial liquid waste and sewage from stud farm (1–3). Recently, several studies have reported the utilization of industrial waste as substrates for the production of single-cell protein (SCP) by growing PTB with wastewater (4–6). Harvested cells of PTB have been used as supplemental feed for fish (7) and laying hens (27).

Accumulated data document that the type of diet affects lipid metabolism and thus, hypercholesterolemia is understood as one of the atherosclerosis-inducing risk...
factors (8). This understanding has engendered current interest in the hypocholesterolemic factors contained in common diets. The dietary component responsible for the cholesterol lowering is generally presumed to be the protein component. Soy protein has received by far the greatest research attention as one of the hypocholesterolemic proteins of plant origin. It shows hypocholesterolemic effects compared with animal proteins in rabbits (9, 10), rhesus monkeys (11), pigs (12), and humans (10).

Consideration has been given to hypocholesterolemic effects of other components in plant food. Among those components studied are carotenoids (13, 14) and nonsaponifiable components (15), fats (16), phospholipids (17), dietary fiber (18), saponins (19), garlic oil (20), and non-protein components of edible mushroom (21). A number of studies have been also conducted on the microbial cells, *Spirulina platensis* (22, 23), *Chlorella* (24), and *Euglena* (25) for their effects on plasma cholesterol and blood pressure in rats.

The purpose of the present work was to study the effects of PTB on cholesterol and triacylglycerol metabolism in the serum and liver of rats fed cholesterol-enriched diets.

**MATERIALS AND METHODS**

*Phototrophic bacteria cells.* *Rhodopseudomonas capsulata* was grown in outdoor culture under natural illumination as previously described (26). The cells of phototrophic bacteria (PTB) were collected by centrifugation and spray-dried. The dry PTB powder contained approximately 9.8% nitrogen and 4.2% carotenoids as determined by the AOAC method (27) and had an ash content of approximately 5.4%.

*Animals and diets.* Male specific-pathogen-free Wistar rats (Shimizu Laboratory Supplies, Kyoto) weighing about 130–150 g at the beginning of the study were separated into three groups. They were housed individually in suspended stainless steel cages in a temperature- (23–25°C) and light-controlled room with a 12-h light-dark cycle beginning at 0700 h with free access to drinking water. The body weight gain was recorded for a 2-day period.

Rats were fed experimental diets for 28 days on a pair-feeding basis by monitoring daily food intake. The composition of the basal diet was as follows (%): casein, 20; DL-methionine, 0.3; alpha-corn starch, 15; cellulose powder, 5; corn oil, 5; AIN-76 mineral mix (36), 3.5; AIN-76 vitamin mix, 1.0; choline bitartrate, 0.2; cholesterol, 1.0; and sucrose, 44. To the basal diet was added 0, 0.2, or 2.0% of PTB at the expense of sucrose.

*Sample collection.* Blood samples was collected between 0900 and 1000 h from the tail vein of unfasted rats under light ether anesthesia. The samples were taken into tubes without anticoagulant and, after standing at room temperature for 1 h, serum was prepared by low-speed centrifugation at 5°C. At the end of the experimental period of 28 days, the rats were killed by ether inhalation, and liver,
kidney, and heart were quickly removed, washed with 0.9% saline, blotted dry on filter paper, and weighed before freezing for storage.

Chemical analysis. Cholesterol, triacylglycerol, and glucose concentrations in the serum were determined enzymatically using commercially available reagent kits, Wako cholesterol Kit No. 274-46401, Wako triacylglycerol Kit No. 274-69802, and Wako glucose Kit No. 273-13901, respectively (Wako Pure Chemical Ind., Osaka). Lipids were extracted from liver with chloroform–methanol (1:2), and assayed for cholesterol and triacylglycerol contents using the same reagent kits as those used for serum.

Statistical analysis. Data were presented as means±SEM. Paired t-tests were used to compare mean difference between the control and experimental groups.

RESULTS

Rat growth, feed intake, and tissue weight

Table 1 compares the feed intake, body weight gain, and tissue weights (liver, kidney, and heart) after 4 weeks of pair-feeding the experimental diets containing PTB at 0% (control), 0.2% (0.2T), or 2.0% (2.0T). The weight gain of rats fed either 0.2T or 2.0T diet was lower than those fed the control diet, but the difference was statistically insignificant. By the diets the weights of kidney and heart did not vary significantly, but the weights of liver differed significantly. The liver weights decreased in proportion to the dietary content of PTB (p<0.05 and p<0.001, respectively).

Cholesterol, triacylglycerol, and glucose concentrations in the serum

The data on serum cholesterol levels are presented in Fig. 1. Supplementation of increasing amounts of PTB induced a significant decrease in the levels of serum cholesterol, which ranged from 115 mg/100ml in rats fed the control diet to 100mg/100ml and 70mg/100ml after 4 weeks in those fed 0.2T and 2.0T diets, respectively. The hypocholesterolemic effect of the 2.0T diet was detected from the early

Table 1. Body weight gain, feed intake, feed efficiency, and organ weights of rats fed diets containing PTB cells.*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.2T</th>
<th>2.0T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g/4 wk)</td>
<td>169±6.8</td>
<td>164±3.7</td>
<td>148±3.4</td>
</tr>
<tr>
<td>Feed intake (g/day)</td>
<td>16.1±1.0</td>
<td>15.6±0.7</td>
<td>15.6±0.7</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.37±0.01</td>
<td>0.37±0.01</td>
<td>0.34±0.01</td>
</tr>
<tr>
<td>Liver (g/100g body wt)</td>
<td>3.2±0.1</td>
<td>2.9±0.1*</td>
<td>2.6±0.1**</td>
</tr>
<tr>
<td>Kidney (g/100g body wt)</td>
<td>0.65±0.01</td>
<td>0.64±0.01</td>
<td>0.62±0.01</td>
</tr>
<tr>
<td>Heart (g/100g body wt)</td>
<td>0.33±0.01</td>
<td>0.32±0.01</td>
<td>0.31±0.01</td>
</tr>
</tbody>
</table>

*Values are means±SEM, n = 4. **Significantly different from the control at p<0.05 and p<0.01, respectively.

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Fig. 1 Serum cholesterol concentrations in rats fed the experimental diets. Each point represents the mean value and the bars represent SEM. n=4. Significantly different from the control at *p<0.05, and **p<0.01.

Fig. 2 Serum triacylglycerol concentrations in rats fed the experimental diets. Each point represents the mean value and the bars represent SEM. n=4. Significantly different from the control at *p<0.05, **p<0.01, and ***p<0.001.

stage of the feeding period and the effect continued during the whole experimental period (p<0.01).

In Fig. 2 is shown the effect of PTB on serum triacylglycerol level. After the 4-wk experimental period, total triacylglycerol in the serum decreased to 40 to 60% of that found in the control diet group.

Table 2 illustrates the HDL-cholesterol levels in the serum of rats at the end of the 4-wk feeding period. The atherogenic indices calculated by using these values were: 1.40, 1.20, and 0.80 for control, 0.2T, and 2.0T diet groups, respectively. The indices showed that the PTB diets were effective in correcting the hypocholesterolemic condition in rats.

Table 3 shows the effect of PTB on serum glucose concentration in rats after 1 and 4 weeks on PTB diets. There were no differences in the glucose content in

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Table 2. Total and HDL-cholesterol concentrations (mg/100 ml) in the serum of rats after 4 weeks on the diets containing PTB cells.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total cholesterol</th>
<th>HDL-cholesterol</th>
<th>Atherogenic index a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>115±5</td>
<td>48±2</td>
<td>1.40±0.15</td>
</tr>
<tr>
<td>0.2T</td>
<td>100±4</td>
<td>45±5</td>
<td>1.20±0.15</td>
</tr>
<tr>
<td>2.0T</td>
<td>70±5*</td>
<td>39±2*</td>
<td>0.80±0.2*</td>
</tr>
</tbody>
</table>

a Value are means±SEM, n=4. b Atherogenic index means the ratio of (VLDL+LDL)-CHOL/HDL-CHOL in serum. *Significantly different from the control at $p<0.01$.

Table 3. Serum glucose concentration (mg/100 ml) in rats fed diets containing PTB cells for 1 and 4 weeks.

<table>
<thead>
<tr>
<th>Diet</th>
<th>1 wk</th>
<th>4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>144±5</td>
<td>147±5</td>
</tr>
<tr>
<td>0.2T</td>
<td>143±4</td>
<td>147±5</td>
</tr>
<tr>
<td>2.0T</td>
<td>138±3</td>
<td>149±4</td>
</tr>
</tbody>
</table>

*Values are means±SEM, n=4.

Table 4. Triacylglycerol and cholesterol concentration (mg/g) in the liver of rats after 4 weeks on the diets containing PTB cells.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Triacylglycerol</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.5±1.6</td>
<td>6.3±0.5</td>
</tr>
<tr>
<td>0.2T</td>
<td>12.7±2.0*</td>
<td>7.3±1.0</td>
</tr>
<tr>
<td>2.0T</td>
<td>12.0±2.4*</td>
<td>7.7±1.5*</td>
</tr>
</tbody>
</table>

*Values are means±SEM, n=4. *Significantly different from the control at $p<0.05$.

serum among the three groups.

Cholesterol and triacylglycerol concentrations in the liver

Table 4 shows the concentration of cholesterol and triacylglycerol in the liver. The PTB diets decreased significantly the triacylglycerol levels in the liver ($p<0.05$), whereas they increased the liver cholesterol level ($p<0.05$).

DISCUSSION

The hypocholesterolemic effects presented here are hitherto unknown for PTB, which exerted significant cholesterol- and triacylglycerol-lowering effects in rat sera. Liver triacylglycerol levels also tended to decrease with PTB feeding. Noticeably, the atherogenic index in the serum of 2.0% PTB diet group was significantly low when compared with that of the casein control group.

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In preliminary experiments of ad libitum feeding regimen, inclusion of PTB cells in the casein-based control diet reduced the feed intake and the growth of rats (data not shown), and this reduced growth might have influenced cholesterol metabolism. However, in the paired-feeding regimen employed in the present study, no demonstrable difference was found in the feed efficiency (weight gain/feed intake) among the three groups. Therefore, the hypocholesterolemic responses to diets containing PTB cells could not be simply attributed to the reduced growth rate.

The accumulated data document that dietary factors affect lipid metabolism and a particular emphasis is given to their association with the risk of atherosclerosis. A hypocholesterolemic effect of dietary factors can be accounted for either by intraluminal or postabsorptive event or by a combination of both events. The intraluminal theory includes the physicochemical interaction of protein hydrolysates with cholesterol and/or bile acid micelles in the intestine during the course of their luminal absorption (28). Recently, the possible participation of the digestion process in the hypocholesterolemic effect of plant protein has frequently been discussed (29–32).

PTB diets increased fecal excretion; average excretion of feces for the 4-wk experimental period was 1.07±0.02, 1.11±0.02, and 1.36±0.03 g/day for control, 0.2T, and 2.0T diet groups, respectively, the difference being statistically significant between the control and 2.0T groups (p<0.05). The increased fecal excretion on PTB feeding may partly be attributed to PTB's poor digestibility. Proteins contained in PTB cells show a low pepsin-pancreatin digestibility. It thus appears that the increased excretion of feces in the 2.0T diet group may be associated, at least in part, with the hypocholesterolemic action of PTB cells.

The postabsorptive theory postulates that plant proteins affect the homeostasis of plasma cholesterol (10,33). One attractive explanation for the hypocholesterolemic effect of PTB involves the specific combination of amino acids. Kritchevsky et al. (34) proposed a relationship between the lysine/arginine ratio of intact protein as a factor involved in the regulation of cholesterolemia. The addition of arginine to casein reduced the cholesterol level in rats (35). Compared with animal protein, PTB protein has a high ratio of arginine to lysine (1.25:1) (26). However, the amount of PTB cells added to the diets accounted for only 1 and 10% of dietary protein, and thus affected the ratio insignificantly.

Carotenoids that originated from the PTB diets were found to have accumulated in the liver of rats to a significant level. The accumulated pigments after being extracted with a chloroform–methanol (2:1) mixture showed a UV absorbance spectrum that resembled those of spirilloxanthin-related substances. The reason why PTB carotenoids accumulate in the liver was not clear, however.

Some carotenoids are known as hypocholesterolemic agents. Amen and Lachance (13) reported that beta-carotene and canthaxanthin have a hypocholesterolemic effect in rats, and beta-carotene seems to displace cholesterol in the transport of lipoproteins. In PTB diet groups, liver cholesterol levels were a little

higher than those of the casein group, while serum cholesterol and triacylglycerol levels were significantly lower. It is thus possible to assume that the carotenoids contained in PTB cells (3.5% in dried cells), although shown to be other than beta-carotene (27), affect the cholesterol transport between the serum and liver.

PTB diets obviously showed a significant hypocholesterolemic effect in rats fed a high cholesterol diet (1% CHOL). Nevertheless, at the present stage of study, it is not clear which factor or factors of PTB cells is responsible for the observed hypocholesterolemic effects. Also uncertain is the effect of components other than the amino acids and carotenoids on the serum cholesterol level. The mechanism involved and the practical implications of our observations remain to be determined.

REFERENCES


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