Hydrolysis of Fish Oil by Marine Bacterial Lipase

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Attempts were made to find marine bacteria which were capable of hydrolyzing *n*-3 polyunsaturated fatty acid-containing fish oils. Out of 45 strains of marine bacteria screened, 3, 6, and 13 strains from genera *Alteromonas*, *Pseudomonas*, and *Vibrio*, respectively, showed the hydrolytic activities of Tween 80 or tributyrin. One strain of genus *Vibrio*, tentatively named VB-5, showed a high activity of hydrolyzing fish oils. The lipase activity from the strain VB-5 was enhanced by adding olive oil into the medium. The olive oil-induced lipase from the strain VB-5 was capable of liberating eicosapentaenoic and docosahexaenoic acids as free fatty acids along with palmitic and oleic acids from fish oils.

Consumption of fish oils has been advocated for antiatherogenic effects attributed to constituent *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.¹⁾ This leads to the attempts to produce *n*-3 PUFA-containing lipids by the lipase reactions of synthesis and interesterification,²⁾ because the lipase is expected to react mildly with *n*-3 PUFA which is labilized by the oxidation. Although a number of microbial lipases from bacteria, fungi, and yeasts are employed for this purpose, satisfactory results are not necessarily obtained. This might result from the low specificity of these microbial lipases toward the *n*-3 PUFA.³⁾

EPA-producing bacteria have recently been isolated from the intestinal contents of marine fishes.⁴⁾ This situation prompted us to search for marine bacterial lipases which have a strong specificity toward *n*-3 PUFA. The present paper deals with the screening of marine bacteria for the production of lipase by which *n*-3 PUFA-containing fish oils are hydrolyzed. It is hoped that the lipase which hydrolyzed fish oils can catalyze the synthetic reaction of *n*-3 PUFA-containing lipids, since the reversible reaction is known for lipase.⁵⁾

Materials and Methods

Fifteen strains of marine bacteria which belong to genera *Alteromonas*, *Pseudomonas*, and *Vibrio*

in each were isolated from the red tides of Tanabe Bay in Wakayama Prefecture of Japan, in 1989. Each strain was cultured in a selected medium, CPY, at 25°C for 4 days. The CPY medium comprised 0.1% casitone, 0.05% proteose peptone, 0.1% yeast extract, 1.3% agar, and 100 ml of 75% natural seawater.

Lipase productivity of each strain was assayed using 1% Tween 80 or 0.75% tributyrin as substrates.^{6,7)} One strain of genus *Vibrio*, tentatively named VB-5, was examined for the hydrolysis of fish oils. The strain VB-5 was cultured in 100 ml of the CPY medium without agar in a 300 ml Erlenmeyer flask on a rotary shaker at 25°C for 5 days. In order to examine whether the lipase was induced by the presence of substrate, 0.75% olive oil was also added to the CPY medium. The growth medium was separated from the cells and the supernatant was used for the assay of lipase activity without purification.

The reaction mixture, composed of 50 mg of triacylglycerol from fish oils, 5 ml of McIlvaine's buffer (pH 7.0), 2.5 ml of 0.05% sodium taurocholate, and 5 ml of supernatant, was incubated at 25°C for 16 h. Air in the reaction flask was replaced by argon gas. After the reaction, fish oils were extracted with ethanol and diethyl ether. The hydrolysis products of fish oils were analyzed quantitatively by thin-layer chromatography (TLC). The TLC plates (Kieselgel 60, ready-made plate from Merck) were developed using *n*-hexane/diethyl ether/formic acid (75:

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25: 2, by volume) and quantitated using a Cosmo F-808 densitometer. Fatty acid composition of each lipid class was also analyzed by gas-liquid chromatography after methylation with methanol containing anhydrous hydrogen chloride.

Results and Discussion

A precipitate derived from oleate or a clear zone due to the hydrolysis of tributyrin was expected to appear around the colonies if the microorganisms secreted the lipases into the CPY medium containing Tween 80 or tributyrin, respectively. Microorganisms capable of precipitating oleate were found in 3, 6, and 13 strains from genera *Alteromonas*, *Pseudomonas*, and *Vibrio*, respectively, when Tween 80 was tested as a substrate of lipase. Almost the same results were obtained by detecting the clear zone due

to the hydrolysis of tributyrin. This result of screening suggests that the strains of genus *Vibrio* have a high ability of producing lipase.

The strain VB-5 was further examined for the hydrolysis of fish oils. The TLC of hydrolysis products demonstrated clearly that fish oils were hydrolyzed by the supernatant solution from the strain VB-5. The lipase activity of the strain VB-5 toward fish oils was enhanced when the strain VB-5 was cultured in the presence of olive oil, suggesting that the lipase from the strain VB-5 was induced by olive oil (Fig. 1). The percentage of triacylglycerol was reduced to 52% by the olive oil-induced lipase, while those of monoacylglycerol, diacylglycerol, and free fatty acid increased to 5, 13, and 23%, respectively. With respect to hydrolyzates of fish oils by the action of olive oil-induced lipase, both 1, 2 (2, 3)-diacylglycerol and 1, 3-diacylglycerol were detected on the TLC plate. This result suggests that the lipase from the strain VB-5 hydrolyzes all ester bonds of triacylglycerol regardless of their positions.

Actual contents of fatty acid in each lipid class were calculated from the data on the compositions of lipid and fatty acid to examine whether the olive oil-induced lipase was capable of hydrolyzing *n*-3 PUFA-containing fish oils (Fig. 2). EPA and DHA as free fatty acids along with

Fig. 1. Hydrolysis products of fish oils by the lipase from the strain VB-5. TG (lane A) from fish oils was incubated in the presence (lanes C and D) or absence (lane B) of the supernatant from the strain VB-5 for 16 h at 25°C. The supernatant for lipase activity was obtained from the strain VB-5 cultured with (lane D) or without (lane C) olive oil. TG, triacylglycerol; FFA, free fatty acid; DG, diacylglycerol; MG, monoacylglycerol.

Fig. 2. Fatty acid content of each lipid class hydrolyzed by olive oil-induced lipase from the strain VB-5. The initial contents of palmitic acid, oleic acid, EPA, and DHA in triacylglycerol (50 mg) as a substrate for lipase assay were 6.2, 6.0, 12.0, and 3.1 mg, respectively. ▨, triacylglycerol; ···, free fatty acid; ▩, diacylglycerol; □, monoacylglycerol.

palmitic and oleic acids were liberated from fish oils by the lipase. These results suggest that the strain VB-5 produced a lipase by which *n*-3 PUFA-containing fish oils were hydrolyzed. Further studies will be reported soon regarding the purification of the lipase from the strain VB-5 and the synthesis of *n*-3 PUFA-containing lipids by this lipase.

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