Polyunsaturated Fatty Glyceride Syntheses by Microbial Lipases

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The degree of glyceride syntheses by lipase TOYO (*Chromobacterium viscosum*) and lipase OF (*Candida cylindracea*) using individual free fatty acids $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{18:4}$, $C_{20:4}$, $C_{20:5}$ and $C_{22:6}$ were compared. Lipase TOYO incorporated each of the fatty acids into glycerol at levels of greater than 89%. Lipase OF incorporated most of the fatty acids at levels above 70% (docosahexaenoic acid incorporation was 63%). It was concluded that these two lipases are feasible for producing glycerides from unsaturated fatty acids.

KEY WORDS: Fatty acid, glyceride, lipase, polyunsaturated, triglyceride.

For therapeutic applications, not only the structures of fatty acids, but also the ways in which they are combined are important (1,2). In previous papers (3,4) we demonstrated that lipase TOYO (*Chromobacterium viscosum*) and lipase OF (*Candida cylindracea*) were effective enzyme preparations for the esterification of γ-linolenic acid, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) with glycerol.

This short communication supplements those results by examining the incorporation of other unsaturated fatty acids into glycerides.

EXPERIMENTAL PROCEDURES

Lipase TOYO (*Chromobacterium viscosum*) was purchased from Toyo Jozo Co., Ltd. (Tokyo, Japan) and lipase OF (*Candida cylindracea*) from Meito Sangyo Co., Ltd. (Nagoya, Japan). Glyceride synthesis was carried out under previously determined conditions (4). To a mixture of 4 mL glycerol and 0.3 mL of individual unsaturated fatty acid (FFA) (all of the FFA used were 99% purity, produced by the Idemitsu petroleum Chemical Ind. Co., Ltd., Tokyo, Japan), lipase solution was added (water content and lipase amount were 0.93% and 200 units, respectively, for Lipase TOYO, and 9.80% and 7650 units, respectively, for Lipase OF). The container was degassed and incubated for 24 hr at 25°C with stirring at 1500 rpm. The reaction was stopped by the addition of 15 mL of ethanol. The proportion of glycerides synthesized was determined by the decrease in acid value. The lipid composition of the recovered material was determined by a thin-layer chromatograph (TLC)-densitometric method (TLC plate, Silica Gel 60,

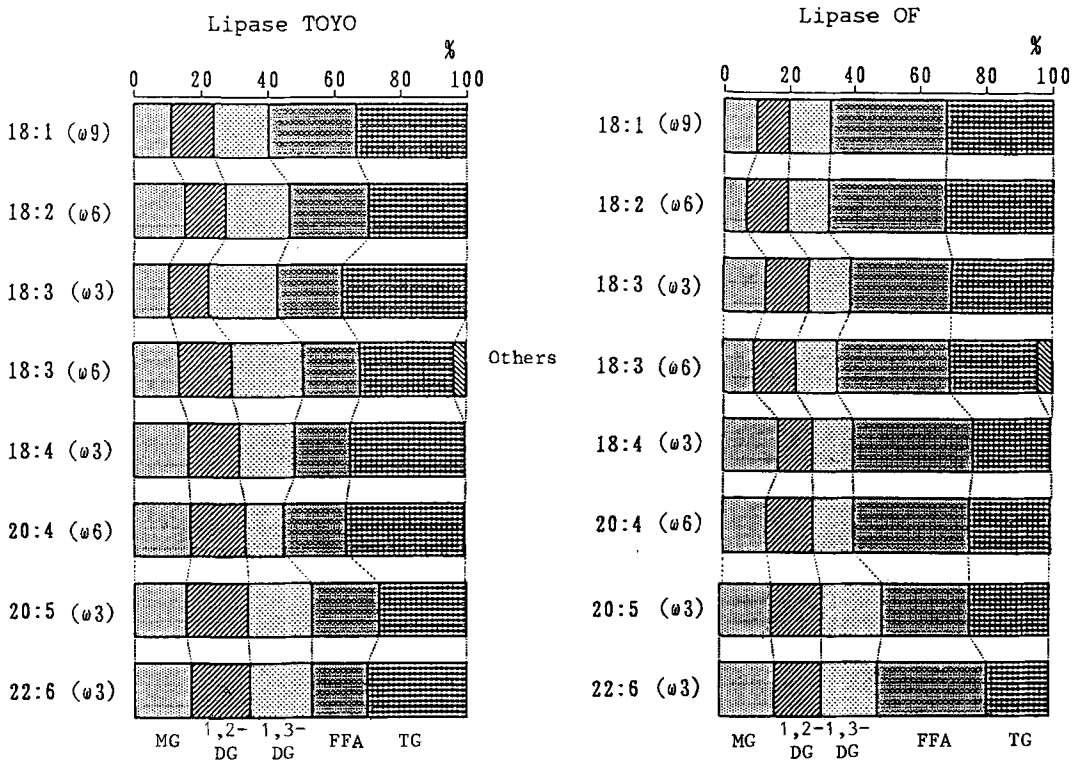

FIG. 1. Overall composition of the oils obtained by reactions catalyzed by lipase TOYO (*Chromobacterium viscosum*) and lipase OF (*Candida cylindracea*).

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TABLE 1

Degree of Esterification of Each Unsaturated Fatty Acid with Glycerol as Determined by Acid Value

Free fatty acid	Degree of incorporation (%)	
	Lipase TOYO	Lipase OF
18:1 (ω9)	95	71
18:2 (ω6)	95	71
18:3 (ω3)	89	74
18:3 (ω6)	94	73
18:4 (ω3)	93	71
20:4 (ω6)	89	73
20:5 (ω3)	91	75
22:6 (ω3)	95	63

Merck, Darmstadt, Germany); developing solvent, n-hexane/ethyl ether/acetic acid (75:25:2, v/v); densitometer, Cosmo F-808 (Tokyo, Japan).

RESULTS AND DISCUSSION

Table 1 shows the degree of glyceride synthesis of the individual unsaturated fatty acids by lipase TOYO and lipase OF. Lipase TOYO incorporated each of the unsaturated FFA into glycerides at levels of 89–95%. As illustrated in Figure 1, triglycerides (TG) made up 26–38% of the recovered lipids, regardless of the FFA used. Lipase OF incorporated over 70% of the unsaturated FFAs into glycerol, except for DHA (DHA was 63%). This lipase produced 18–33% of TG (Fig. 1). DHA is less susceptible to esterification when catalyzed by lipase OF as compared to lipase TOYO. Residual FFA was less in lipase TOYO catalyzed reactions. In general, the results illustrated in Figure 1 indicate that FFAs with higher carbon numbers or the numbers of double bonds produced less TG. However, this does not mean that the initial incorporation of FFA into glycerol decreases when the carbon number or number of double bonds increases, because the proportions of residual FFA were nearly constant. Further work on the mechanisms of the reactions might help determine the effects of chain length and double bond numbers on the composition of the products, but we conclude that these two lipases should be feasible for producing glycerides consisting of almost any kind of unsaturated fatty acids.

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