Enzymatic Syntheses of Structured Triacylglycerols

Tsuneo YAMANE
Laboratory of Molecular Biotechnology
Graduate School of Bio- & Agro-Sciences
Nagoya University, Nagoya 464-8601, JAPAN.

1. Introduction

Currently research of enzyme biotechnology related to lipids is actively being carried out worldwide with an expectation of contribution to fats and oils industry. Notably enzymatic syntheses of so called structured triacylglycerols (sTAGs, structured lipids) are major research topics in this area.

Structured triacylglycerols are in the broadest sense defined as any triacylglycerols (TAGs) that have been restructured to change positions of fatty acids (FAs) and modified to change the FA compositions from the native state. From the viewpoint of chemistry, natural edible fats and oils are merely mixtures of a number of triacylglycerols that are different in terms of both FA species and their distribution along the glycerol backbone. In contrast to natural edible lipids, sTAGs are TAGs modified either chemically or enzymatically in either the type of FA or the position of the FAs. In the strictest sense, the term sTAG is given to a TAG with particular fatty acid (FA) at specific position of glycerol hydroxy moieties. According to the strictest definition, we have classified sTAGs as shown in Table 1.[1]

Table 1. Classification of structured triacylglycerols (sTAGs)

Number of different FA	Type	Chirality	Stereoisomers
monoacid-	AAA	achiral	
diacid-	ABA	achiral	
	ABB, BBA	chiral	enantiomers
triacid-	ABC, CBA	chiral	enantiomers
	BCA, ACB	chiral	enantiomers
	CAB, BAC	chiral	enantiomers

Note that in Table 1, FAs (A, B, and C) are shown in the order of their located positions *sn*-1, *sn*-2, and *sn*-3 of glycerol backbone.

sTAGs containing polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ALA), have become of great interest because of various pharmacological effects of these FAs. These include several health benefits on cardiovascular desease, immune disorders and inflammation, renal disorders, allergies, cancer, etc. These FAs may be also essential for brain and retina development in humans.

Among sTAGs containing PUFA, sTAGs containing one molecule PUFA and two molecules of medium-chain fatty acids (MCFAs) are very noticeable because of their high intestinal absorption rate.

sTAG can be synthesized either chemically or enzymatically. However enzymatic synthesis of sTAG is more advantageous over chemical ones because enzymes are generally specific giving rise to less or no byproducts, and exhibit catalytic actions under mild condition (PUFAs are very unstable. They are prone to be easily isomerized, oxidized and polymerized). We have recently studied lipase-catalyzed syntheses of sTAG containing PUFA from the viewpoints of both monitoring the reactions and increasing the yield.[2]

2. Monitoring the Reactions

For the production of a targeted sTAG, it must be known which types of TAG are formed and how many FAs are incorporated at a specific hydroxy position of glycerol. For example, when one wants to produce pure 1-eicosapentaenoyl 2,3-diocatanoylglycerol [(EPA)C8C8, a ABB type sTAG in Table 1] by lipase-catalyzed transesterification reaction between eicosapentaenoic acid ethyl ester (EPAEE) and tricaprylin by the following reaction scheme:

$$\text{EPAEE} + \text{Tricaprylin} \longrightarrow \text{(EPA)C8C8 [and/or C8C8(EPA)]} + \text{C8EE} \qquad [1]$$

A number of (about 11) chemical species may appear during the reaction. We succeeded in separating and identifying all these chemical species by applying silver ion high pressure liquid chromatography.[3,4] We made it possible to separate directly (EPA)C8C8 [and/or C8C8(EPA)] and C8(EPA)C8 by using 2-propanol as a modifier in hexane-acetonitrile based mobile phase and contriving its gradient program for silver-ion HPLC.

3. Increasing the Yield

As is the case with most enzymatic reactions, the performance of enzymatic syntheses of pure sTAG containing PUFA depends on many factors involving type of reaction, enzyme and its immobilization, temperature, water content, composition of substrates, physical properties of the substrates, reaction time, mode of operation, etc. Among them, important factors affecting yield of sTAG by enzymatic synthesis are substrates ratio, thermodynamic shift, side reactions (hydrolysis and acyl migration), and water content. Solvent-free reaction system is also a crucial factor that should be taken into consideration in view of industrial production circumstance.[5]

We have studied recently the reaction [1] in solvent-free system under the water activity (a_w) control.[3,4,6] As the best result, molar yield of ca. 90% of the targeted sTAG was obtained after 16 h.[6]

Pure ABA type sTAG can be produced by several methods. We reported a chemoenzymatic synthesis of C8(EPA)C8.[7] More recently we have developed a novel two-step enzymatic process that seems more promising as shown in the following scheme:[8]

$$\text{Glycerol} + 3\text{EPAEE} \longrightarrow (\text{EPA})(\text{EPA})(\text{EPA}) + C_2H_5OH \qquad [2]$$

$$(\text{EPA})(\text{EPA})(\text{EPA}) + \text{C8EE} \longrightarrow \text{C8(EPA)C8} + 2\text{EPAEE} \qquad [3]$$

The first step [2] is esterification in solvent-free system with a non-regiospecific lipase. The reaction mixture from the step[2] is subjected to the second step[3] without any purification after separation of the immobilized enzyme. The second step[3] is transesterification again in solvent-free system with a 1,3-regiospecific lipase. By careful a_w control in the step [2] and applying vacuum (reduced pressure) in the step 2 [3], an overall yield of the targeted product throughout the two steps reached ca. 70%.[8]

Recent achievements in these high-yield enzymatic syntheses of various types of sTAGs will attract industrial people's attention. The processes will be hopefully implemented at large-scale in order to supply these sTAGs in food and/or in pharmaceutical industries as functional foods and/or medicines, respectively.

4. References

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