NOTE

Porcine Pancreatic Lipase Hydrolysis of Synthetic Highly Unsaturated Triacylglycerols

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Highly unsaturated triacylglycerols (TAGs) compried of icosapentaenoic (EPA) and docosahexaenoic acids (DHA) were chemically synthesized and assessed for their hydrolyzability toward porcine pancreatic lipase. Triicosapentaenoylglycerol (EEE) and tridocosahexaenoylglycerol (DDD), both unsaturated TAGs, were hydrolyzed to a lesser extent than trilinolenoylglycerol (LnLnLn), trilinoleoylglycerol (LiLiLi) or tripalmitoylglycerol (PPP). The hydrolyzability of synthetic TAGs followed the order, LiLiLi>LnLnLn>PPP≫DDD>EEE. EEE was the most resistant to pancreatic lipase hydrolysis.

Key words : docosahexaenoic acid, hydrolysis, icosapentaenoic acid, pancreatic lipase, triacylglycerol

1 Introduction

Icosapentaenoic acid (EPA, 20 : 5) and docosahexaenoic acid (DHA, 22 : 6) present in marine oils are expected as functional foods and medicine because of their specific physiological effects against thrombosis, cholesterol buildup and allergies. In general, ethyl ester of EPA and DHA or native fish oil is utilized as drugs and foods. Recently, chemical and enzymatical preparations of triacylglycerol (TAG) type of EPA- or DHA-rich oils are tried from marine oils[1)~5)]. These oils contain highly unsaturated TAGs which provide two or three moles of EPA and DHA in a TAG molecule. Some studies[6),7)] have started for the oxidative stability of the highly unsaturated TAGs. We practically observed that fish oils containing lots of highly unsaturated TAGs were very susceptible to autoxidation[7)]. However, there are few nutritional data, *eg.* digestion and absorption for highly unsaturated TAGs.

The purpose of this study is to determine the *in vitro* pancreatic lipase hydrolysis of several highly unsaturated TAGs comprised of EPA and DHA which we chemically synthesized.

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2 Experimental

Tridocosahexaenoylglycerol (DDD), triicosapentaenoylglycerol (EEE), 1,2 (or 2,3)-diicosapentaenoyl-3(or 1)-palmitoylglycerol (EEP) and 3(or 1)-icosapentaenoyl-1,2 (or 2,3)-dipalmitoylglycerol (PPE) were chemically synthesized on the basis of the method of Awl *et al.*[8)] Trilinolenoylglycerol (LnLnLn), trilinoleoylglycerol (LiLiLi) and tripalmitoylglycerol (PPP) were purchased from Sigma Chemical Co. (St. Louis, U. S. A.) as well as porcine pancreatic lipase (EC : 3. 1. 1. 3). All TAGs were purified through the Florsil column. The purity of TAGs was more than 95% by HPLC analysis.

Ten milligrams of TAGs were mixed to 0.4 mL of 1% sodium taurocholate solution, 1.0 mL of 45% $CaCl_2$ solution and 16.6 mL of 0.25 M Tris-HCl buffer (pH 8.0) containing 5% arabic gum. Porcine pancreatic lipase solution (4 mg or 20 mg/2 mL) was added to the TAG mixture and stirred at 37℃ for 30 min. To quench the hydrolysis, *n*-hexane/ethanol/70% H_2SO_4 (300 : 200 : 1, vol/vol/vol) mixture was added to the reaction solution. *n*-Hexane fraction containing hydrolyzed TAGs was collected by centrifugation. Hydrolysate was measured by TLC-FID method[9)] using *n*-hexane/diethyl ether/

formic acid (80 : 20 : 1, vol/vol/vol) as a mobile phase. Hydrolyzability of TAGs was determined by residual amounts of TAGs after the reaction. Experiments were performed triplicate.

3 Results and Discussion

Hydrolyzability of seven kinds of synthetic TAGs to porcine pancreatic lipase is shown in **Table−1**. EEE and DDD being typical highly unsaturated TAGs provided very poor hydrolyzability (below 10 %), while TAGs comprising palmitic (16 : 0), linoleic (18 : 2) or linolenic acids (18 : 3) were easily hydrolyzed. The hydrolyzability of TAGs was LiLiLi > LnLnLn > PPP ≫ DDD > EEE, and did not always follow the unsaturation ratio of TAGs. Especially, EEE showed the significantly low hydrolyzability (18 %) at the high level of lipase (20 mg to 10 mg oil), while hydrolyzability of DDD was 34%. Among EPA-containing TAGs, EEP and PPE were very easily hydrolyzed in comparison with EEE. Considerable hydrolyzability of EEP and PPE is due to the release of palmitic acid from the corresponding TAGs. Increasing the EPA content in TAGs resulted in less hydolysis of TAGs (PPP>PPE>EEP>>EEE).

In this study, highly unsaturated TAGs containing EPA and DHA were chemically synthesized and then subjected to pancreatic lipase hydrolysis. As a result, it was found that the hydrolyzability of highly unsaturated TAGs depended on the varieties of TAGs as well as fatty acids, and that EEE and DDD were very resistant to pancreatic lipase hydrolysis. Bottino *et al.*[10] found that EPA-and DHA-containing TAGs in whale oil were less hydrolyzed by pancreatic lipase, and they postulated that the resistance of EPA-and DHA-containing TAGs was due to the steric hindrance effect of EPA and DHA. Tanaka *et al.*[11] also reported that DDD was most resistant to *Candida* lipase hydrolysis among DHA-rich TAGs. Our results were also similar to those observation. In previous papers[12),13)], we found that unusual TAGs comprising branched-chain fatty acids such as tris(2-methylpalmitoyl) glycerol and tris(2-ethylpalmitoyl) glycerol were resistant to porcine pancreatic lipase hydrolysis, *in vitro* and *in vivo*. The remarkable resitance of EEE to pancreatic lipase hydrolysis might be due to both the steric hindrance of EPA and the specific TAG molecule structure. From these results, it is suggested that EEE and DDD might be a non-digestible fat and that they might hardly exhibit physiological effects of EPA and DHA *in vivo*. It is necessary to determine the *in vivo* digestibility and absorptibility of EPA-and DHA-rich oils containing highly unsaturated TAGs.

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Table−1 Hydrolyzability of synthetic highly unsaturated triacylglycerols to pancreatic lipase.

Triacylglycerol	Lipase (mg/10 mg oil)	Hydrolyzability* (%)
Tripalmitoylglycerol (PPP)	4	59.7±3.0
Trilinoleoylglycerol (LiLiLi)	4	93.0±1.8
Trilinolenoylglycerol (LnLnLn)	4	69.4±3.2
Tridocosahexaenoylglycerol (DDD)	4	8.3±2.0
	20	33.7±0.6
Triicosapentaenoylglycerol (EEE)	4	5.6±1.0
	20	17.7±3.0
1,2 (or 2,3)-Diicosapentaenoyl-3 (or 1)-palmitoylglycerol (EEP)	4	46.7±0.3
3 (or 1)-Icosapentaenoyl 1,2 (or 2,3)-dipalmitoylglycerol (PPE)	4	54.6±2.7

* Mean±SD

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合成高度不飽和トリアシルグリセリンの豚すい(膵)リパーゼ加水分解性

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イコサペンエタン酸(EPA)やドコサヘキサンエン酸(DHA)を含む高度不飽和トリアシルグリセリンを合成し，豚膵臓リパーゼに対する加水分解性を測定した。トリイコサペンタエノイルグリセリン(EEE)やトリドコサヘキサエノイルグリセリン(DDD)のような高度不飽和トリアシルグリセリンは，トリリノレノイルグリセリン(LnLnLn)やトリリノレオイルグリセリン(LiLiLi)，トリパルミトイルグリセリン(PPP)に比べ加水分解されにくかった。合成トリアシルグリセリンの加水分解性はLiLiLi>LnLnLn>PPP>>DDD>EEEの順で，とくにEEEは膵リパーゼによる加水分解に対し強い抵抗性を示した。
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