Short Paper

Triarachidonin and Diarachidonoylphosphatidylcholine in "Ogonori" *Gracilaria verrucosa*

Kuninori Kinoshita,*1 Koretarō Takahashi,*1 and Kōichi Zama*1

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Lipid of plants and animals have their own characteristic fatty acid composition. In aquatic animals, dominant unsaturated fatty acids (UFA) are represented by eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), while in the case of aquatic plants such as sea weeds, predominant UFA is considered to be represented by EPA alone that accounts for 2–47% of the total fatty acid.[1] DHA seldom appears in most cases. From this aspect, *G. verrucosa* is considered to have an extraordinary fatty acid composition. Takagi and Itabashi*2 reported that the prominent fatty acid of *G. verrucosa* is arachidonic acid that in case occupies over 60% of compositional fatty acids. Therefore, lipid metabolism of *G. verrucosa* is of great interest. The objective of this study is to investigate the distribution of arachidonic acid in lipid classes for the first step of elucidating the characteristic lipid metabolic system of *G. verrucosa*.

Sample was collected at Taisei-cho, Hokkaido, Japan in May and Sep., 1983 and at Shinori coast, Hakodate, Japan in June, July and Sep., 1984. Total lipid was extracted according to the modified method of Bligh and Dyer. Triglyceride (TG) and phosphatidylcholine (PC) were separated by silicic acid column chromatography and purified by preparative thin layer chromatography (TLC).[2,3] The purified TG was fractionated into individual molecular species by reversed phase high performance liquid chromatography (HPLC).[2] PC was hydrolyzed with phospholipase C (EC 3.1.4.3, *Clostridium perfringens*) according to the method of Renkonen.[4] Hydrolyzed lipid *i.e.* diglyceride was purified by preparative TLC and acetylated according to the modified method of Privett and Nutter.[5] The glyceride acetate that represents the acyl combination of PC was also fractionated into individual molecular species by HPLC.[3] The analysis of fractionated peak components was done by gas liquid chromatography.[2,3] Amounts of TG and PC were calculated by the densitometric method.

Small variations in lipid content were observed among the five collected samples, ranging from 0.2% to 0.4% on a wet basis. In contrast to this, lipid composition showed significant variations. For example TG ranged from 18.3% to 33.0% and PC ranged from 0.3% to 19.8% of the total lipid. Arachidonic acid was extremely rich in all lipid classes of all the sample examined. In the case of TG, arachidonic acid accounted for 22.4–44.2%. Therefore, high probability of the occurrence of simple TG (triarachidonin) as well as simple PC (diarachidonoylphosphatidylcholine) was suggested.

In fact, HPLC analysis of TG and PC molecular species demonstrated the extraordinary amount of triarachidonin and diarachidonoylphosphatidylcholine as illustrated in Fig. 1 and Fig. 2. Among the five collected samples, triarachidonin ranged from 20.4% to 44.2% and diarachidonoylphosphatidylcholine ranged from 56.2% to 64.2%.

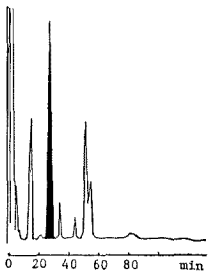

Fig. 1. Representative HPLC chromatogram of TG. Equipment: HITACHI 638-50, column: 4×300 mm, Chemcosorb I-5C18, Flow rate: 0.5 ml/min, Eluting solvent: acetone/acetonitrile (3: 2, v/v), Ditector: Shodex RI, Sensitivity: 10×8, Chart speed: 2.5 mm/min.

Fig. 2. Representative HPLC chromatogram of diglyceride acetate from PC. Equipment: HITACHI 638-50, Column: 4×300 mm, Chemcosorb I-5C18, Flow rate: 1.0 ml/min, Eluting solvent: acetonitrile/water (100: 1, v/v), Ditector: Shodex RI, Sensitivity: 10×8, Chart speed: 2.5 mm/min.

Recently, the occurrence of prostagrandin E_2, A_2 in *G. verrucosa* was pointed out by Fusetani and Hashimoto[6]. It is of great interest whether the simple TG and PC such as triarachidonine or diarachidonoylphosphatidylcholine are susceptable of attack by hydrolytic enzymes and whether the released arachidonic acid enters the "arachidonic acid cascade" by lipoxygenase or cycloxygenase. This would be discussed elsewhere.

M. Muraoka contributed experimental skills to the program.

References

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*1 Laboratory of Food Chemistry I, Faculty of Fisheries, Hokkaido University, Minatochō, Hakodate 041, Japan (木下邦則, 高橋是太郎, 座間宏一: 北海道大学水産学部).

*2 T. Takagi and Y. Itabashi: Abstracts of the 22th annual meeting of Japan Oil Chem. Soc., Osaka, 1983, pp. 144 (In Japanese).