Short Paper

Separation of Polyunsaturated Triglycerides by Column Chromatography on Silicic Acid

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It has been shown that eicosapentaenoic acid (20:5 ω 3) may play an important role for mammals as an agent to reduce blood platelet aggregation, thereby lowering the risk of heart attacks.¹⁾ It was also recognized that absorption of 20:5 ω 3 in a triglyceride (TG) form was more advantageous than in an ethyl ester form in human subjects.²⁾ We have recently demonstrated that the scallop hepatopancreas is a potential source of 20:5 ω 3.^{3,4)} In this report, I describe the preparative scale isolation of 20:5 ω 3-highly enriched TG from the hepatopancreas lipids of scallops by using a silicic acid column chromatographic technique.

Crude lipids obtained from the scallop hepatopancreas by the method reported previously⁴⁾ yielded 5.1% of the wet tissue weight, consisting of 54.0% TG which had 39.2% 20:5 ω 3. An aliquot (5.0 g) of the tissue lipids was dissolved in a small volume of *n*-hexane and applied to a column (200 g, ϕ 3.8 $\times$ 43 cm) of silicic acid (Wakogel C-200)-celite 545 (2:1 w/w, Wako Pure Chemical Industries Ltd, Tokyo) which was activated for 5 h at 110°C and packed with *n*-hexane. The column was developed with a solvent series of *n*-hexane (500 ml) and 5% diethyl ether-*n*-hexane (3500 ml). Fractions of 500 ml were collected and determined by thin-layer chromatography. Six fractions containing TG were eluted with 5% diethyl ether-*n*-hexane (Table 1).

As given in Table 1, the TG fractions showed the various mobilities in TLC, varying from R_f 0.32 to 0.49. These TG were differentiated from each other on TLC by the degree of unsaturation of the component fatty acids. This result was in agreement with that reported previously by Mangold and Malins.⁵⁾ The TG fractions consisted of more saturated fatty acids for frs. 1 and 2, and of polyunsaturated fatty acids for frs. 3, 4, and 5. This was specially true for the TG in frs. 3, 4, and 5 which contained a large

amount of 20:5 ω 3, corresponding to about 50, 57, and 57%, respectively. These three fractions accounted for 37% of the original TG in the tissue lipids, thus the 20:5 ω 3 in TG being concentrated to 1.3–1.4-fold. The proportion of 21:5 ω 3 in the original TG (6.1%), comparable to that in the gastropod mollusc (4.5%),⁶⁾ was elevated to about 7 to 8% for the TG in frs. 4 and 5. Fraction 6 contained considerably large amounts of both 20:5 ω 3 and 16:4. Finally, the crude lipids from the hepatopancreatic tissues (100 g) gave two fractions containing 0.6 g and 0.4 g of light yellowish colored polyunsaturated TG with 50% and 57% 20:5 ω 3, respectively. Their combined fractions totalled 1.0 g TG containing 52% 20:5 ω 3 and accounted for 20% of the original hepatopancreas lipids. For separating fish oil TG molecules according to the degree of unsaturation, Dolev and Olcott⁷⁾ have used a column of silica gel impregnated with silver nitrate and Laakso *et al.*⁸⁾ utilized silver ion high-performance liquid chromatography. Silicic acid column chromatography used in this study is a rapid and low cost technique in separating polyunsaturated TG and applicable for a large scale production of these compounds from the hepatopancreas lipids of scallops.

References

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Table 1. Elution sequence and fatty acid composition of fractionating triglycerides from the hepatopancreas lipids of scallops by silicic acid column chromatography

	TG in tissue lipid	Fraction in TG					
		1	2	3	4	5	6
Yield	mg ^{*1}	367	1302	597	268	146	6
	% ^{*2}	7.3	26.0	11.9	5.4	2.9	0.1
	% ^{*3}	13.7	48.5	22.2	10.0	5.4	0.2
R _f value ^{*4}		0.42	0.49	0.45	0.41	0.37	0.32
Fatty acid % ^{*5}							
14:0	5.7	10.4	6.7	4.3	2.4	1.2	0.2
16:0	10.0	21.3	12.9	5.5	2.3	1.3	3.2
16:1	10.2	15.9	12.8	7.9	4.4	2.2	3.0
18:1	5.4	12.2	7.1	2.2	1.0	0.7	1.7
16:4	1.0	0.2	0.2	1.8	3.1	6.9	23.2
20:5 ω 3	39.2	16.2	35.7	49.7	56.7	56.6	34.8
21:5 ω 3	6.1	1.7	3.5	5.6	7.0	7.8	6.0
22:6 ω 3	3.7	3.8	3.7	4.0	3.9	3.3	2.8
Saturates	19.2	36.7	23.0	12.3	7.1	5.1	7.1
Monoenes	19.2	33.9	23.8	13.7	8.8	6.5	9.4
Polyenes	61.3	29.2	53.0	73.8	84.0	88.1	83.5
Branched	0.3	0.2	0.2	0.2	0.1	0.3	
Total U ^{*6}	310.1	169.8	275.4	365.6	410.0	423.3	378.5

*1 Each fraction contained triglycerides separated by silicic acid column chromatography.

*2 % to the hepatopancreas lipids of scallops.

*3 % to total triglycerides in the hepatopancreas lipids.

*4 Data were obtained by TLC on silicic acid plates using developing solvent such as *n*-hexane-diethyl ether-acetic acid (85:15:1, v/v/v).

*5 Expressed as weight percentage of total fatty acids.

*6 Unsaturation index = fatty acid double bond $\times$ %.

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