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Short Paper

High level of 6,9,12,15,18,21-tetracosahexaenoic acid found in lipids of Ophiuroidea *Ophiura sarsi* Lütken

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6,9,12,15,18,21-tetracosahexaenoic acid (24:6n-3, THA) is a type of very long chain fatty acid belonging to n-3 polyunsaturated fatty acids (PUFA). It was already known that THA occurred in the lipids of several species of echinoderms[1] or coelenterates[2] and in their predators.[3,4] THA is expected to have beneficial functions for human health, as for other PUFA. Recently, we determined that THA isolated from a brittle star, *Ophiura sarsi* Lütken has anti-inflammatory and anti-allergic properties similar to those of docosahexaenoic acid (22:6n-3).[5] During this study, we observed that the level of THA contained in the lipids of the brittle star was higher than those reported for other echinoderms. In this paper we describe the THA content in each lipid class of the brittle star.

The samples used in this study were caught at a depth of 123–243 m in Wakasa bay in November 1994 and they were kept at −20°C for 8 months prior to analysis. The mean body weight of 226 individuals used was 1.1 g and the total lipids content, extracted by the method of Bligh and Dyer,[6] was 0.5%.

The components of the total lipids from the brittle star were separated with a Sep-Pak silica cartridge column (Millipore Co., Milford, MA, USA) according to the method of Hamilton and Comai.[7,8] The separated components were submitted to thin layer chromatography and were identified by comparing their Rf values with those of the standard. The thin layer chromatography was conducted using the developing solvent consisting of *n*-hexane: diethyl ether: acetic acid (80:20:1, v/v) for neutral lipids and chloroform: methanol: H_2O (40:20:2.5, v/v) for polar lipids.

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As shown in Table 1, the total lipids consisted of the mixture of steryl ester and hydrocarbon (1.4%), triacylglycerol (29.6%), the mixture of free fatty acid, monoacylglycerol, and diacylglycerol (5.9%), phosphatidylcholine (31.7%), phosphatidylethanolamine (22.9%), and sterol (8.6%). The mixture of free fatty acid, monoacylglycerol and diacylglycerol was assumed to be the degradation products produced during frozen storage, although the degradation products contained very small amounts of monoacylglycerol and diacylglycerol and the majority being free fatty acid. It was, therefore, suggested that the degradation products were mainly derived from phospholipids rather than triacylglycerol.

In order to determine the fatty acid composition, the total lipids and the separated components were esterified with hydrochloric acid-methanol and were submitted to gas chromatography using 10% DEGS (Chromosorb WAW) packed column. The kinds of fatty acids found in the total lipids were similar to those of other echinoderms as previously reported. Major fatty acid compositions of the total lipids and the separated components are shown in Table 1. The major fatty acids of the total lipids were 16:0 (8.6%), 18:1 (11.4%), 20:1 (9.9%), 20:5n-3 (15.5%) and THA (13.9%). The THA content in the total lipids from this brittle star was significantly higher than those from other echinoderms (less than 10%) according to any previous reports,[1] whereas the percentage of 22:6n-3 was considerably lower than that of THA. Comparing the THA content in each lipid class, the THA contents in phosphatidylcholine and phosphatidylethanolamine were around 20%, while that in triacylglycerol was less than 10%. It was already reported that THA was concentrated in phospholipids rather than triacylglycerols in other echinoderms.[1] The concentration of THA in phosphatidylcholine and phos-

Table 1 Major fatty acids composition of the total lipids and the separated components of the brittle star (%)

Lipid class	TL[1]	SE + HC[1] (1.4%)	TG[1] (29.6%)	FFA + MG + DG[1] (5.9%)	PC[1] (31.7%)	PE[1] (22.9%)	S[1] (8.6%)[2]
Fatty acid							
14:0	7.4	6.5	8.6	5.7	Tr.[3]	8.1	
16:0	8.6	9.8	11.0	6.9	4.4	6.2	
16:1	4.6	4.1	5.9	3.0	1.9	1.6	
18:0	4.8	2.5	2.6	4.3	7.1	4.0	
18:1	11.4	10.9	11.4	7.1	4.1	13.0	
20:1	9.9	15.4	10.9	4.8	11.0	12.4	
20:4n-6 + 22:1	6.4	10.4	7.6	7.5	5.0	5.4	
20:5n-3	15.5	3.1	9.4	28.2	15.9	24.5	
22:6n-3	2.6	1.4	4.1	4.1	1.4	1.9	
24:6n-3	13.9	3.0	8.4	22.7	24.3	18.9	
Others	14.9	32.9	20.1	5.7	24.9	4.0	

[1] TL, total lipids; SE + HC, the mixture of steryl ester and hydrocarbon; TG, triacylglycerol; FFA + MG + DG, the mixture of fatty acids, monoacylglycerol, and diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; S, sterol.
[2] % of total lipids.
[3] Tr., trace (less than 0.1%).

phatidylethanolamine in the brittle star was, however, more remarkable than those in phospholipids of other echinoderms. We also determined the fatty acid composition of the total lipids from a starfish belonging to Echinodermata caught in the same area as the brittle star and with a diet of detritus similar to the brittle star. The percentage of THA in the starfish was 1.2% at most (detailed data not shown). This result supported Takagi *et al*.[1] who suggested that THA in Crinoidea and Ophiuroidea did not originate from the diet.

Since the brittle star *Ophiura sarsi* is widely distributed with a high density in the upper bathyal zone around Japan,[9] it is considered useful as a resource of THA.

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