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Level and Composition of Diacyl Glyceryl Ethers in the Different Tissues and Stomach Contents of Giant Squid *Moroteuthis robusta*

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The lipid class compositions of the different tissues and stomach contents from the giant squid *Moroteuthis robusta* of the northwestern Pacific Ocean were investigated.

In this species, the liver contained a remarkably large amount of lipids, which were characterized by a high percentage of triglycerides and diacyl glyceryl ethers. Conversely, the above types of lipids were not detectable in the flesh and gonad lipids, but were high in phospholipids. In addition, lipids from the stomach contents also consisted of large amounts of diacyl glyceryl ethers. Glyceryl ethers derived from diacyl glyceryl ethers of the liver and stomach contents were very similar in composition. The most abundant component was chimyl alcohol followed by selachyl alcohol. Fatty acids of diacyl glyceryl ether and/or triglycerides in the liver and stomach contents lipids were similar in composition, respectively. Also discussed are the fatty acid compositions of the total lipids obtained from the examined body tissues and stomach contents.

In most marine organisms, triglycerides generally exist as the principal reserves of storage lipids. Cephalopod species, such as the common squid *Todarodes pacificus*, also have been found to possess a large amount of these compounds in the liver.[1] However, some peculiar species of deep-sea nektonic squids, Cranchiidae sp.[2] and Oegopsidae sp.,[3] are reported to contain wax esters in relatively large proportions within their lipids. On the other hand, the authors have demonstrated previously that three species of gonatid squids belonging to the family Gonatidae: *Berryteuthis magister*,[1,4-6] *Gonatopsis makko*,[1] and *G. borealis*,[7] all contained in their livers significant amounts of glyceryl ethers in the form of diacyl glyceryl ethers. Subsequently, it was found that a giant squid belonging to the family Onychoteuthidae, *Moroteuthis robusta*, also contained in its liver considerable amounts of these types of lipids.

The present investigation deals with the lipid class compositions of the flesh, liver, and gonad, and the component glyceryl ethers of the liver of *M. robusta*. In regard to the possible origins of the above ether-linked lipids, the compositions of the lipid class and/or glyceryl ethers and fatty acids in diacyl glyceryl ethers of the stomach contents from this squid were examined and compared. The fatty acid compositions of total lipids obtained from the examined tissues and stomach contents were also determined.

Materials and Methods

Materials

Two specimens of the giant squid *M. robusta* were caught by drift gill net at lat. 47°59′N. and 48°19′N., and long. 173°26′E. and 172°59′E. of the northwest Pacific Ocean in August, 1987 and 1989 respectively. They were kept frozen at −20°C until analyzed. The sizes of two specimens were 102 cm and 110 cm in mantle length, 11.7 kg and 17.5 kg in body weight, 1.0 kg and 1.3 kg in liver weight, 71 g and 178 g in gonad weight, and 242 g and 74 g in stomach contents weight, respectively. Samples of the flesh, liver, gonad, and stomach contents were used for lipid extraction.

Lipid Extraction and Analysis

Extraction of tissue lipids, quantitative determination of constituent lipids by the thin-layer chromatography-flame ionization detector methods, isolation of GE and fatty acids from the tissue lipids, preparation of isopropylidene derivatives of GE and methyl esters of fatty acids, and the determination of the above compounds by gas-liquid chromatography (GLC) were performed

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as described in a previous report.[7] The mass spectra of isopropylidene derivatives of glyceryl ethers were recorded using a JEOL JMS-HX 110 instrument equipped with a glass column (1.5 m × 3.2 mm id.) of Unisole 3000 on Uniport C (80/100 mesh). The mass spectrometer was operated at 23 eV.

Results and Discussion

Lipid Class Composition

Lipid contents and class compositions of the examined tissues and stomach contents of the giant squid *M. robusta* appear in Table 1. Also included in Table 1 are the compositions of unsaponifiable materials isolated from the total lipids of the above samples.

The livers from the two specimens of *M. robusta* contained 16.7–18.6% lipids. These percentages were much higher than those of the flesh (0.5–0.6%), gonad (0.9–1.2%), and stomach contents (2.2–3.9%). The lipids of livers were characterized by relatively high levels of triglycerides (51.2–68.2%) and diacyl glyceryl ethers (13.8–18.5%). In comparison, the flesh and gonad lipids were only traced, or not detected at all in the above types of lipids, but were high in phospholipids (81.1–86.2%). Additionally, the lipids from the stomach contents also consisted of large amounts of diacyl glyceryl ethers (38.8–68.3%).

On the other hand, the unsaponifiable materials of liver lipids were dominated by glyceryl ethers and sterols. These accounted for 35.0–46.9% and 48.6–62.4% respectively. However, those of the flesh and gonad were composed predominantly of sterols (83.7–97.5%). Also minute amounts of fatty alcohols were detected in unsaponifiable materials of the liver and gonad lipids; 0.8% and 0.2%, respectively. In particular, the unsaponifiable materials of the stomach contents were characteristically rich in glyceryl ethers (46.2–75.2%).

Irrespective of different sampling sites for the two *M. robusta* specimens, both their lipid contents and class compositions for the examined tissues and stomach contents were very similar to one another. It was found that the giant squid *M. robusta* as well as gonatid squids *B. magister*,[1,4–6] *G. makko*[1] and *G. borealis*[7] were peculiar species of cephalopod, which contained in the liver considerable amounts of glyceryl ethers in the form of

Table 1. Lipid content and class composition of each different tissue and stomach contents of *M. robusta*

		Specimen 1				Specimen 2			
		Flesh	Liver	Gonad	Stomach contents	Flesh	Liver	Gonad	Stomach contents
Lipid content	%*1	0.6	18.6	1.2	3.9	0.5	16.7	0.9	2.2
Lipid component	%*2								
SE			8.0		7.9		8.2		14.7
DAGE			18.5		68.3		13.8		38.8
TG			51.2	tr*4	0.6	tr	68.2		22.4
ST		17.9	1.6	13.6	3.0	17.2	1.3	13.1	0.9
PL		81.1	8.2	83.8	7.3	81.6	7.7	86.2	22.4
Others*3		1.0	12.5	2.6	12.9	1.2	0.8	0.7	0.8
Unsaponifiable material content	%*2	17.7	15.2	13.5	32.1	16.0	14.8	13.5	23.6
Unsaponifiable material component	%*5								
FA			0.8	0.2					
ST		96.6	48.6	83.7	22.6	96.9	62.4	97.5	51.7
GE		0.1	46.9	1.7	75.2	0.4	35.0	0.4	46.2
Others*6		3.3	3.7	14.4	2.2	2.7	2.6	2.1	2.1

*1 % to wet wt. basis of tissue.
*2 % to total lipid.
*3 Consisted of fatty acid and partial glyceride.
*4 Trace (less than 0.05%).
*5 % to unsaponifiable material.
*6 Consisted of hydrocarbon and polar compound.
SE, steryl ester; DAGE, diacyl glyceryl ether; TG, triglyceride; ST, sterin; PL, phospholipid; FA, fatty alcohol; GE, glyceryl ether.

Table 2. Composition of glyceryl ethers in the liver and stomach contents lipids of *M. robusta*

	Specimen 1		Specimen 2	
	Liver	Stomach contents	Liver	Stomach contents
Component*1		Peak area %		
14:0	4.9	4.0	3.4	3.7
15:0	1.4	1.0	1.1	1.1
16:0	53.8	52.5	57.0	55.9
17:0	0.1	0.3	0.3	0.3
18:0	2.7	2.4	2.3	2.0
14:1	0.1	tr*2	tr	tr
16:1	1.1	0.8	0.4	0.9
17:1	0.1	tr		
18:1	24.0	23.7	26.1	25.2
19:1	tr	tr	tr	tr
20:1	8.4	10.2	6.8	8.2
22:1	1.5	3.4	0.9	1.1
15:0br*3	0.2	0.1	tr	tr
16:0br	0.1	0.1	0.1	0.1
17:0br	0.2	0.2	0.3	0.2
18:0br	0.4	0.4	0.5	0.5
19:0br	0.4	0.4	0.3	0.4
20:0br	0.6	0.5	0.4	0.3
Saturates	62.9	60.2	64.1	63.0
Monoenes	35.2	38.1	34.2	35.4
Branched	1.9	1.7	1.7	1.6

*1 Indicated by chain length and double bond of alkyl moiety.
*2 Trace (less than 0.05%).
*3 Branched compound.

diacyl glyceryl ethers. In addition, it is of interest to note that the lipids from the stomach contents of *M. robusta* had remarkably high amounts of diacyl glyceryl ethers. This differs from those of *B. magister*[6] and *G. borealis*,[7] both which were rich in wax esters.

Glyceryl Ether Composition

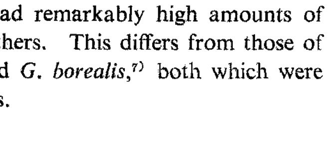
The quantitative distribution of glyceryl ether components from the liver and stomach contents of *M. robusta* is given in Table 2. In GLC-MS analysis, the main components of isopropylidene drivatives of glyceryl ethers were found to give the following characteristic peaks: molecular ion (M^+), M^+-15 (CH_3), and *m/e* 101 , base peak. The characteristics of the above components are described as follows. Even numbers of carbon atoms, ranging from C_{14} to C_{22}, prevailed for the alkyl moiety. The most predominant component found was chimyl alcohol (16:0, 53.8–57.0% for liver and 52.5–55.9% for stomach contents) followed by selachyl alcohol (18:1, 24.0–26.1% and 23.7–25.2%), indicating a high percentage of saturates (62.9–64.1% and 60.2–63.0%). Branched glyceryl ethers also occurred, but only in small amounts (1.7–1.9% and 1.6–1.7%).

Table 3. Composition of fatty acids of each different tissue and stomach contents lipids of *M. robusta*

	Specimen 1				Specimen 2			
	Flesh	Liver	Gonad	Stomach contents	Flesh	Liver	Gonad	Stomach contents
Component*1				Peak area %				
14 : 0	3.5	2.6	3.5	3.7	2.5	1.7	1.6	1.6
16 : 0	21.5	12.0	27.8	4.3	19.8	12.5	16.7	8.7
17 : 0	0.6	2.1	1.7	2.2	0.7	3.2	1.0	1.4
18 : 0	4.4	2.8	14.0	0.7	4.7	3.2	8.7	2.3
16 : 1	0.9	3.9	1.5	4.4	1.0	3.7	0.8	3.8
18 : 1	4.7	22.4	10.3	16.9	5.7	23.6	5.8	23.1
20 : 1	10.6	17.7	14.4	16.5	11.5	15.1	8.2	14.3
22 : 1	0.8	12.9	1.0	22.6	1.3	10.6	0.4	10.9
24 : 1	tr*2	1.7	0.1	2.6	0.2	2.4	0.2	2.6
18 : 2ω6	tr	1.6	0.2	1.2	0.1	1.3	0.3	1.3
20 : 4ω6	2.7	0.8	2.1	0.8	2.7	1.2	3.6	1.4
20 : 5ω3	18.1	5.2	11.3	7.3	18.1	6.1	27.1	10.1
22 : 6ω3	28.5	9.1	5.5	10.8	25.6	9.5	16.1	12.5
Saturates	30.4	19.9	47.7	11.2	28.0	21.0	28.2	14.4
Monoenes	17.7	59.4	28.4	63.4	20.9	56.6	17.2	55.9
Polyenes	51.9	20.4	23.8	25.0	51.0	22.4	54.6	29.6
Branched	tr	0.3	0.1	0.4	0.1			0.1

*1 No. of carbon atom: no. of double bond.
*2 Trace (less than 0.05%).

The glyceryl ethers in the liver of *M. robusta* were similar in composition to those of the gonatid squids *B. magister*,[1,4-6] *G. makko*,[1] and *G. borealis*[7] reported previously. It should be noted that the glyceryl ethers of the liver lipids of these giant or gonatid squid species, these being characteristically rich in chimyl alcohol, were extremely different from those of the liver lipids of ratfishes and sharks. The component glyceryl ethers for ratfishes and sharks are rich in selachyl alcohol (54–65%).[8-11]

Fatty Alcohol Composition

The characteristics of minute amounts of the fatty alcohol components in the liver of *M. robusta* are described as follows. Carbon atoms with an even number, ranging from C_{14} to C_{24}, were more abundant than odd and branched ones. The main components were 22:1 (33.5%), 16:1 (16.2%), 16:0 (14.7%), 24:1 (11.1%), 20:1 (9.6%), 18:1 (4.5%), 23:1 (2.6%), 14:0 (1.9%), 18:0 (1.8%), and 21:1 (1.4%) alcohols, indicating a high monoenoic content (79.6%). Also, the branched components occurred in small amounts (1.5%).

In marine organisms, the fatty alcohols were important precursors in the biosynthesis of the alkyl chain of glyceryl ethers.[12,13] However, in the liver of *M. robusta*, the composition of glyceryl ethers rich in chimyl alcohol was different from that of fatty alcohols rich in the 22:1 alcohol component.

Fatty Acid Composition of Total Lipids, Diacyl Glyceryl Ethers, and Triglycerides

Table 3 shows the major fatty acid composition of the total lipids from the examined tissues and stomach contents of the giant squid. As for the examined samples, 16:0, 18:0, 18:1, 20:1, 22:1, 20:5ω3, and 22:6ω3 acids were characteristic. However, within that generalization differences did occur. Of the flesh and/or liver of specimens 1 and 2, the component fatty acid percentages were very similar to one another. These consisted predominantly of polyenes (51.0–51.9%) for the former, and of monoenes (56.6–59.4%) for the latter. While the fatty acid composition of the gonad from specimen 2 was markedly different from that of specimen 1, the former tended to be more polyenoic with an abundance of 20:5ω3 and 22:6ω3 acids. Specimen 1 was more saturated with 16:0 and 18:0 acids, possibly on the basis of the tissue development. On the other hand, the component fatty acid percentages of the stomach

Table 4. Composition of fatty acids of diacyl glyceryl ethers and triglycerides in the liver and stomach contents lipids of *M. robusta*

	Specimen 1			Specimen 2			
	Liver		Stomach contents	Liver		Stomach contents	
	DAGE	TG	DAGE	DAGE	TG	DAGE	TG
Component*1				Peak area %			
14:0	6.6	2.6	3.6	1.3	2.1	1.6	2.8
16:0	5.8	15.5	3.4	6.8	15.8	5.6	12.4
17:0	1.6	2.0	2.6	0.7	1.3	1.2	1.5
18:0	2.0	3.2	0.9	2.1	3.4	1.1	2.2
16:1	4.0	3.7	5.1	3.2	4.1	3.4	5.5
18:1	21.7	23.8	19.2	25.9	25.9	28.5	26.1
20:1	20.2	18.4	18.0	16.2	15.1	15.4	14.1
22:1	15.7	12.9	23.7	10.5	11.1	12.7	13.0
24:1	2.1	1.6	2.7	2.5	2.0	2.0	2.2
18:2ω6	1.6	1.6	1.8	1.3	1.2	1.5	1.4
20:4ω6	0.6	0.6	0.6	1.0	0.8	1.0	0.7
20:5ω3	3.5	3.0	5.4	7.7	4.4	7.8	7.1
22:6ω3	8.3	6.3	6.8	14.4	6.9	11.8	5.5
Saturates	16.5	23.8	11.0	11.2	22.9	9.8	19.4
Monoenes	64.8	61.4	69.6	59.1	59.1	62.8	62.2
Polyenes	18.1	14.7	18.6	29.5	16.8	26.8	18.3
Branched	0.6	0.1	0.8	0.2	0.2	0.6	0.1

*1 No. of carbon atom: no. of double bond.
DAGE, diacyl glyceryl ether; TG, triglyceride.

contents were characterized by large proportions of monoenes (55.9–63.4%). These were rich in 18:1, 20:1, and 22:1 acids.

Fatty acid compositions of diacyl glyceryl ethers and triglycerides isolated from the liver and stomach contents lipids of *M. robusta* (specimens 1 and 2) are given in Table 4. In both specimens, the fatty acid compositions of diacyl glyceryl ethers consisted predominantly of monoenes (59.1–64.8%) with 18:1, 20:1, and 22:1 acids. These were very similar in composition to that of the stomach contents (62.2–69.6%). Also, triglycerides of the liver were similar in component fatty acid percentages to those of the stomach contents. They consisted largely of monoenes (59.1–61.9% for the former, 62.2% for the latter) with 18:1, 20:1, and 22:1 acids.

The metabolism of ether-linked lipids in squid, especially in relation to hydrolysis, adsorption, and synthesis, is not well known. Based on the fact that diacyl glyceryl ethers were found at considerably high concentrations in the liver and/or stomach contents obtained from both *M. robusta* specimens, it is postulated that the ether-linked lipids in the liver may have originated either directly or indirectly from dietary sources.

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