Positional Distribution of 24:6(n-3) in Triacyl-*sn*-Glycerols from Flathead Flounder Liver and Flesh

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This paper presents the positional distribution of very long-chain fatty acids, 24:6(n-3), in triacyl-*sn*-glycerols (TG) of flathead flounder (*Hippoglossoides dubius*). Each of the liver and flesh TGs was subjected to the stereospecific analysis. The liver TGs contained 24:6(n-3) at concentrations of 1.5, 1.2 and 1.7 mole % in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively, and the flesh TGs had 9.0, 7.8 and 7.1 mole % in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively. This fatty acid was distributed almost evenly among the three positions of the TGs. No preference for the *sn*-2 position was observed in contrast to the general tendency for the distribution of longer-chain polyunsaturated fatty acids, such as 22:6(n-3), 22:5(n-3) and 20:5(n-3). There was essentially no difference in the positional distributions of the liver and flesh TGs. The results obtained in this study give new fundamental information to the investigation of very long-chain fatty acids.

KEY WORDS: Flathead flounder, positional distribution, stereospecific analysis, tetracosahexaenoic acid [24:6(n-3)], triacyl-*sn*-glycerols, very long-chain fatty acids.

Tetracosapolyenoic acids, belonging to a family of very long-chain fatty acids, have been found in the lipids of several marine organisms, e.g., marine fish (1–3), sea lilies (4), brittle stars (4) and marine coelenterates (5). Our recent study (Ota, T., Y. Chihara, Y. Itabashi and T. Takagi, unpublished data) showed that the lipids of flathead flounder were rich in all-*cis*-6,9,12,15,18,21-tetracosahexaenoic acid [24:6(n-3)]. The lipids contained this fatty acid at concentrations of 6–9% of total fatty acids in flesh, 3% in liver and 6% in viscera. The primary origin of 24:6(n-3) in the flathead flounder lipids is assumed to be the diet, but its role remains obscure.

In the flathead flounder, 24:6(n-3) was abundant in triacyl-*sn*-glycerols (TG), rather than in the phospholipids. This fact prompted us to research the positional distribution of this fatty acid in TGs. There have been several reports on positional distributions of fatty acids in fish TGs (6–9). The distributions of 22:6(n-3), 22:5(n-3) and 20:5(n-3) were detailed in these reports. However, there have been no data for the positional distribution of 24:6(n-3) in fish TGs.

In this study, we have carried out the stereospecific analyses of the TGs isolated from liver and flesh of flathead flounder, and, in these, we have determined the positional distributions of 24:6(n-3) and other fatty acids. The data obtained should be useful as further information to confirm the origin of 24:6(n-3) and to clarify its function.

MATERIALS AND METHODS

Materials. Male flathead flounders (*Hippoglossoides dubius*) used were caught in the Sea of Japan at latitude 44° 50′N, longitude 139° 54′E, in February 1990. The fish were an average of 30 cm in length and 245 g in weight per head. The liver and flesh were separated from the fish and kept frozen at −25°C until used.

Isolation of TGs. Total lipids were extracted from the liver and flesh of each fish by the method of Bligh and Dyer (10). TGs were isolated from the other lipids by column chromatography on Kiesel Gel 60 (Merck, Darmstadt, Germany) with chloroform for elution, and subsequently by preparative thin-layer chromatography (TLC) on Kiesel Gel 60G plates (0.5 mm thickness; Merck) with *n*-hexane/diethyl ether/acetic acid (80:20:1, vol/vol/vol) for development.

Preparation of fatty acid methyl esters. Fatty acid methyl esters were prepared by heating 1–2 mg of TGs in a mixture of 1,2-dichloroethane (0.6 mL), methyl acetate (25 μL) and 1M sodium methoxide–methanol solution (25 μL) at 50°C for 2 h. After adding acetic acid (6 μL) and removing the solvents, the products were taken up in *n*-hexane.

Gas–liquid chromatography (GLC). GLC analyses of the methyl esters were performed on a Shimadzu GC-9A gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a flame-ionization detector and a WCOT column (50 m × 0.25 mm i.d., 0.2-μm film thickness) coated with Silar 5CP. The column temperature was 195°C, and injector and detector temperatures were 240°C. Hydrogen was the carrier gas. Peak area percentages were measured with a Shimadzu C-R6A integrator.

Stereospecific analysis of TGs. The method for stereospecific analysis of fish TGs (9) was used after modifications as follows. TGs (10 mg), mixed with trinonadecanoylglycerol (0.5 mg), were partially hydrolyzed with ethyl magnesium bromide in dry diethyl ether (0.33 mL of 0.33 M solution) for 25 s before adding 0.1 mL of acetic acid/diethyl ether (1:9, vol/vol) and 1 mL of water. All of the products were immediately reacted with 3,5-dinitrophenyl isocyanate (50 mg) in dry toluene (1.0 mL), in the presence of dry pyridine (0.1 mL), overnight at ambient temperature. Resulting di-3,5-dinitrophenylurethane derivatives of 1- and 2-monoacylglycerols were isolated by preparative TLC on Kiesel Gel 60G plates (0.25-mm thickness) with chloroform/acetone (96:4, vol/vol) for development. The 1-monoacylglycerol derivatives were resolved into *sn*-1- and *sn*-3-monoacylglycerol fractions by high-performance liquid chromatography with a Shimadzu LC-6A isocratic pump, a Hitachi L-4200 ultraviolet spectrophotometric detector (Hitachi Co., Tokyo, Japan) and a Shimadzu C-R6A integrator. Two columns of Sumichiral OA-4100 (25 cm × 4 mm i.d., 5-μm particles; Sumitomo Chemical Co., Osaka, Japan) in series were used with *n*-hexane/1,2-dichloroethane/ethanol (40:12:3, vol/vol/vol) as mobile phase at a flow rate of 0.5 mL/min at −10°C. Detection was at 254 nm. Each monoacylglycerol derivative was converted to fatty acid methyl esters and analyzed by GLC in a manner similar to that used for the analysis of fatty acids from TGs.

RESULTS AND DISCUSSION

Fatty acid composition of TGs. Table 1 shows the fatty acid compositions of TGs from liver and flesh of flathead flounder. The principal fatty acids, at more than 2 mole %

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TABLE 1

Fatty Acid Composition of Triacyl-*sn*-Glycerols from Male Flathead Flounder (mole %)

Fatty acid	Liver	Flesh	Fatty acid	Liver	Flesh
12:0	0.04	0.10	22:1(n-7)	0.11	0.22
iso-14:0	0.01	0.12	24:1(n-9)	0.54	0.65
14:0	5.88	12.21			
4,8,12-TMTD[a]	0.08	0.14	16:2(n-4)	0.91	0.98
iso-15:0	0.70	0.65	16:3(n-4)	0.73	0.45
anteiso-15:0	0.05	0.22	16:4(n-1)	0.02	0.16
15:0	0.31	0.72	18:2(n-6)	0.27	0.66
iso-16:0	0.16	0.25	18:3(n-6)	0.07	0.20
Pristanic	0.03	0.06	18:3(n-3)	0.05	0.25
16:0	18.29	12.81	18:4(n-3)	0.07	0.47
anteiso-17:0	0.21	0.20	18:4(n-1)	0.03	0.07
iso-18:0	0.20	0.31	20:2 5,11	0.05	0.14
18:0	1.55	3.32	20:2 5,13	0.06	0.15
20:0	0.05	0.24	20:2(n-6)	0.09	0.19
			20:3 5,11,14	0.07	0.17
14:1(n-7)	0.04	0.06	20:3(n-6)	0.03	0.17
16:1(n-9)	0.35	0.34	20:4(n-6)	0.39	1.17
16:1(n-7)	15.09	5.53	20:3(n-3)	0.08	0.13
16:1(n-5)	0.39	0.62	20:4(n-3)	0.15	0.37
18:1(n-13)	0.57	0.92	20:5(n-3)	2.76	10.11
18:1(n-9,11)	35.61	12.68	22:2 7,15	0.03	0.08
18:1(n-7)	4.37	3.88	21:5(n-3)	0.14	0.36
18:1(n-5)	0.62	0.78	22:4(n-6)	0.12	—
19:1(n-8)	0.10	0.06	22:5(n-6)	0.03	0.09
20:1(n-15)	0.15	1.48	22:5(n-3)	0.52	0.83
20:1(n-11,13)	1.99	7.59	22:6(n-3)	0.84	2.67
20:1(n-9)	1.40	1.30	24:6(n-3)	1.44	7.86
20:1(n-7)	0.49	1.01			
20:1(n-5)	0.15	0.54	Total saturates	27.56	31.35
22:1(n-11,13)	0.90	1.97	Total monounsaturates	63.49	40.92
22:1(n-9)	0.62	1.29	Total polyunsaturates	8.95	27.73

[a]TMTD, trimethyltridecanoic acid.

of the total fatty acids, in either TG were 14:0, 16:0, 18:0, 16:1(n-7), 18:1(n-9), 18:1(n-7), 20:1(n-11,13), 20:5(n-3), 22:6(n-3) and 24:6(n-3). The liver TGs contained larger amounts of 16:1(n-7) (15.1 mole %) and 18:1(n-9) (35.6 mole %) than the flesh TGs, whereas the flesh TGs showed higher proportions of 14:0 (12.2 mole %), 20:1(n-11,13) (7.6 mole %) and 20:5(n-3) (10.1 mole %) than the liver TGs. The 24:6(n-3) contents of the liver and flesh TGs were 1.4 and 7.9 mole % to the total fatty acids, respectively. This fatty acid was much more concentrated in the flesh TGs than in the liver TGs. The content of total polyunsaturated fatty acids was much higher in the flesh TGs (27.7 mole %) than in the liver TGs (9.0 mole %). The proportions of 24:6(n-3) in the total polyunsaturated acids were 16 mole % in the liver TGs and 28 mole % in the flesh TGs.

Positional distribution of fatty acids in TGs. Table 2 shows the positional distribution of fatty acids in TGs from the liver and flesh. The liver TGs contained 24:6(n-3) at concentrations of 1.5, 1.2 and 1.7 mole % in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively. In the liver TGs, 14:0 and 16:0 were preferentially esterified in the *sn*-2 position, while 18:0 showed a slight preference for the *sn*-1 position. Monounsaturated acids, 16:1(n-7), 18:1(n-9) and 18:1(n-7), were primarily associated with both the *sn*-1 and *sn*-3 positions; 20:1(n-11) and 20:1(n-9) with the *sn*-3 position; and 22:1(n-11) and 22:1(n-9) with the *sn*-1 position. Of the polyunsaturated acids, 20:5(n-3) was preferentially located in the *sn*-3 position, followed by the *sn*-1 and *sn*-2 positions, whereas 22:6(n-3) was esterified in the *sn*-1 and *sn*-2 positions, followed by the *sn*-3 position.

In the flesh TGs, the contents of 24:6(n-3) were 9.0, 7.8 and 7.1 mole % in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively. Of the saturated acids, 14:0 and 16:0 were preferentially esterified in the *sn*-2 position, whereas 18:0 was abundant in the *sn*-3 position. Of the monounsaturated acids, 16:1(n-7) and 18:1(n-9) were distributed in the three positions without significant selectivity, and 18:1(n-7) was primarily associated with the *sn*-1 and *sn*-3 positions. Longer-chain monounsaturated acids, 20:1(n-11), 22:1(n-11) and 22:1(n-9), were preferentially located in the *sn*-1 position, followed by the *sn*-3 and *sn*-2 positions. Of the polyunsaturated fatty acids, 20:5(n-3) was more concentrated in the *sn*-3 position, followed in sequence by the *sn*-2 and *sn*-1 positions, whereas 22:6(n-3) showed essentially no preference for a particular position.

Brockerhoff *et al.* (6) pointed out the general tendency of longer-chain polyunsaturated acids to be preferentially esterified in the *sn*-2 position in fish TGs. Litchfield (7,8) showed essentially that about two-thirds of 22:6(n-3) and 22:5(n-3) were esterified in the *sn*-2 position. In the recent report by Ando *et al.* (9), 50–85% of 22:6(n-3) and 22:5(n-3) were located in the *sn*-2 position.

The general tendency cannot be extended to include the case of 24:6(n-3). Assignments of 24:6(n-3) to the *sn*-1, *sn*-2 and *sn*-3 positions were 35, 27 and 38%, respectively,

TABLE 2

Positional Distribution of Fatty Acids in Triacyl-*sn*-Glycerols from Male Flathead Flounder (mole %)

	Liver			Flesh		
Fatty acid	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3
12:0	0.02	0.09	0.04	0.07	0.03	0.18
iso-14:0	0.01	0.03	0.02	0.09	0.19	0.09
14:0	3.47	11.21	2.64	9.36	18.53	9.45
4,8,12-TMTD[a]	0.06	0.12	0.06	0.20	0.19	0.05
iso-15:0	0.61	0.87	0.61	0.59	0.87	0.52
anteiso-15:0	0.02	0.05	0.04	0.32	0.32	0.05
15:0	0.20	0.52	0.22	0.68	1.16	0.40
iso-16:0	0.10	0.23	0.14	0.27	0.36	0.14
Pristanic	0.02	0.03	0.04	0.02	0.10	0.05
16:0	10.51	35.61	7.73	11.25	16.43	11.21
anteiso-17:0	0.18	0.32	0.12	0.27	0.23	0.13
iso-18:0	0.24	0.20	0.12	0.47	0.26	0.22
18:0	1.92	1.59	1.13	2.35	2.49	4.79
20:0	0.04	0.09	0.02	0.25	0.19	0.27
14:1(n-7)	0.02	0.09	0.04	0.04	0.10	0.04
16:1(n-9)	0.20	0.58	0.22	0.29	0.55	0.21
16:1(n-7)	22.38	8.90	14.31	5.58	4.14	6.66
16:1(n-5)	0.54	0.38	0.26	1.02	0.49	0.41
18:1(n-13)	0.85	0.44	0.44	1.67	0.32	0.81
18:1(n-9,11)	37.60	21.79	47.91	11.14	12.38	14.22
18:1(n-7)	4.50	2.55	6.15	4.59	2.33	4.59
18:1(n-5)	0.57	0.41	0.89	0.50	1.49	0.45
19:1(n-8)	0.12	0.09	0.12	0.07	—	0.11
20:1(n-15)	0.10	0.20	0.14	2.46	0.52	1.49
20:1(n-11,13)	1.72	1.45	2.80	11.80	4.91	6.46
20:1(n-9)	1.15	1.02	2.08	1.40	1.65	0.95
20:1(n-7)	0.38	0.44	0.65	0.16	0.87	1.81
20:1(n-5)	0.18	0.09	0.18	0.99	0.45	0.25
22:1(n-11,13)	1.57	0.32	0.87	3.89	0.94	1.29
22:1(n-9)	0.83	0.32	0.69	2.19	0.71	1.06
22:1(n-7)	0.18	0.06	0.12	0.72	—	—
24:1(n-9)	1.27	—	0.38	1.88	—	0.20
16:2(n-4)	1.05	0.76	0.97	0.48	1.42	1.04
16:3(n-4)	0.69	1.10	0.42	0.50	0.94	0.02
16:4(n-1)	—	0.03	0.02	0.11	0.29	0.09
18:2(n-6)	0.26	0.26	0.28	0.57	0.58	0.81
18:3(n-6)	0.06	0.09	0.06	0.20	—	0.36
18:3(n-3)	0.01	0.06	0.04	0.18	0.29	0.27
18:4(n-3)	0.04	0.12	0.08	0.50	0.52	0.41
18:4(n-1)	0.01	0.06	0.01	—	—	—
20:2 5,11	—	—	0.18	—	—	0.36
20:2 5,13	0.06	0.09	0.06	—	0.19	0.23
20:2(n-6)	0.02	0.17	0.04	0.11	0.29	0.16
20:3 5,11,14	0.10	—	0.14	—	—	0.45
20:3(n-6)	0.02	0.03	0.04	0.11	0.16	0.21
20:4(n-6)	0.15	0.93	0.12	1.02	0.87	1.54
20:3(n-3)	0.04	0.12	0.08	0.36	—	0.05
20:4(n-3)	0.14	—	0.34	—	—	0.97
20:5(n-3)	2.50	2.55	3.51	6.92	9.51	13.20
21:5(n-3)	0.10	0.23	0.08	—	—	0.93
22:4(n-6)	0.26	—	0.10	—	—	—
22:5(n-3)	0.28	1.07	0.20	0.72	1.42	0.45
22:6(n-3)	1.13	1.01	0.40	2.69	2.49	2.82
24:6(n-3)	1.52	1.19	1.65	8.95	7.83	7.07
Total saturates	17.40	51.00	12.93	26.19	41.35	27.55
Total monounsaturates	74.16	39.13	78.25	50.39	31.85	41.01
Total polyunsaturates	8.44	9.87	8.82	23.42	26.80	31.44

[a]See Table 1 for definition.

in the liver TGs, and 38, 33 and 30%, respectively, in the flesh TGs. In this study, 24:6(n-3) was distributed almost evenly among the three TG positions and showed no preference for the *sn*-2 position. This result indicates that the general tendency for longer-chain polyunsaturated fatty acids cannot be applied to 24:6(n-3). In addition, the general tendency was not found to hold for 22:6(n-3) in the flathead flounder. Less than 40% of 22:6(n-3) was esterified in the *sn*-2 position in the liver and flesh TGs. On the other hand, 22:5(n-3) showed a distribution similar to the general tendency, i.e., 69 and 55% of this acid were located in the *sn*-2 position of the liver and flesh TGs, respectively, and much lower proportions were esterified in the *sn*-1 and *sn*-3 positions. In this study, 20:5(n-3) showed the preference for the *sn*-3 position rather than the *sn*-2 position. Although the positional distribution of 20:5(n-3) has been reported to resemble that of 22:6(n-3) and 22:5(n-3) (6–9), several instances of the preferential location in the *sn*-3 position were also reported recently (9).

Comparison between the liver and flesh TGs. It is possible that the TGs originating from secretory tissues, such as liver and intestine, may represent the true specificities of the synthetic process, whereas the storage form may be the product of synthesis from different pools (11). When the positional distributions of fatty acids are compared between the liver and flesh TGs (Table 2), 16:0, 18:0, 16:1(n-7), 18:1(n-9), 20:1(n-11), 20:1(n-9) and 22:6(n-3) showed that the distribution noted in the liver TGs was essentially lost in the flesh TGs. It is probable that the positional distributions of these fatty acids in TGs are different among the liver and other secretory tissues, and the TGs originating from the liver may be less accumulated in the flesh. In fish, 18:1 and 16:1 are metabolized faster than the other fatty acids (12–14), although they can be biosynthesized *de novo* by fish (15).

In contrast, distributions of 24:6(n-3) were almost consistent between the liver and flesh TGs. This consistency suggests the common positional specificity of 24:6(n-3) in the liver and other secretory tissue TGs and/or much accumulation of TGs originating from the liver. Similarly, 14:0, 18:1(n-7), 22:1(n-11), 22:1(n-9), 20:5(n-3) and 22:5(n-3) also showed the almost consistent distribution between the liver and flesh TGs. Because most of these fatty acids were of dietary origin (15), 24:6(n-3) is also presumed to be of dietary origin, as revealed in our recent work.

This study reported the positional distribution of 24:6(n-3) in the liver and flesh TGs of flathead flounder. The positional distribution of 24:6(n-3) was characterized by low positional specificity among the *sn*-1, *sn*-2 and *sn*-3 positions, and consistency of the specificity between the liver and flesh TGs. On the other hand, 20:5(n-3) showed higher specificity for the *sn*-3 position in the liver and flesh TGs, and low positional specificity of 22:6(n-3) in the flesh TGs was not observed in the liver TGs. Very long-chain fatty acids with more than C24 chainlength have been of special interest during recent years (16,17). The results obtained in this study provide fundamental information to investigations of such fatty acids.

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REFERENCES

1. Linko, R.R., and H. Karinkanta, *J. Am. Oil Chem. Soc. 47*:42 (1970).
2. Golovnya, R.V., T.E. Kuzmenko, V.P. Uralets and A.L. Samusenko, *Prikl. Biokhim. Mikrobiol. 14*:609 (1978).
3. Ackman, R.G., and J.C. Sipos, *J. Fish. Res. Bd. Canada 21*:841 (1964).
4. Takagi, T., M. Kaneniwa and Y. Itabashi, *Lipids 21*:430 (1986).
5. Svetashv, V.I., A.A. Popkov and M.V. Vysotskii, *Biochim. Biophys. Acta. 1083*:161 (1991).
6. Brockerhoff, H., R.J. Hoyle, P.C. Hwang and C. Litchfield, *Lipids 3*:24 (1968).
7. Litchfield, C., *Ibid. 3*:417 (1968).
8. Litchfield, C., *Fette Seifen. Anstrichm. 75*:223 (1973).
9. Ando, Y., K. Nishimura, N. Aoyanagi and T. Takagi, *J. Am. Oil Chem. Soc. 69*:417 (1992).
10. Bligh, E.G., and W.J. Dyer, *Can. J. Biochem. Physiol. 37*:911 (1959).
11. Breckenridge, W.C., in *Handbook of Lipid Research*, edited by A. Kuksis, Plenum Press, New York, 1978, p. 223.
12. Pande, S.V., and J.F. Mead, *J. Biol. Chem. 243*:352 (1968).
13. Bilinski, E., and R.E.E. Jonas, *J. Fish. Res. Bd. Canada 27*:857 (1970).
14. Murata, H., and T. Higashi, *Bull. Japan. Soc. Sci. Fish. 45*:211 (1979).
15. Ackman, R.G., in *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil*, edited by S.M. Barlow, and M.E. Stansby, Academic Press, London, 1982, pp. 41–55.
16. Tomáš Řezanka, T., *Prog. Lipid Res. 28*:147 (1989).
17. Morita, N., N. Okajima, M. Gotoh, H. Hayashi, H. Okuyama and S. Sasaki, *Arch. Microbiol. 157*:223 (1992).

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