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Prediction of Relative Retention Value of the Individual
Molecular Species of Diacyl Glycerolipid on
High Performance Liquid Chromatography

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Abstract

The relative retention value of the individual molecular species of acetyldiglyceride derived from phosphatidylcholine on reverse phase high performance liquid chromatography was considered to be dependant principally on the addition theorem of chemical potentials of the two fatty acid residues.

It was demonstrated that the chemical potential of each fatty acid residue is equivalent to the relative retention potential of each fatty acid residue; and that the addition of the relative retention potentials of the two fatty acid residues denoted the logarithm of the relative retention value, the relative retention time of each individual molecular species of acetyldiglyceride.

Martin formulated the formulae[1] :

$$\Delta\mu_B / R \cdot T = \Delta\mu_A / R \cdot T + \Delta\mu_X / R \cdot T \tag{1}$$

$$\log(\alpha_B/\alpha_A) = \Delta\mu_X / R \cdot T \tag{2}$$

where A and B are members of a homologous series differing by the functional group X ; $\Delta\mu_X$ is the difference in chemical potential of the group X in the chromatographic system. R is the gas constant; and T is the absolute temperature. If we consider α_A as the partition coefficient of the standard molecular species and α_B as the partition coefficient of each molecular species, α_B/α_A will correspond to the relative retention value, the relative retention time (RRT) of each molecular species. So from formula [2],

$$\log (\alpha_B/\alpha_A) = \log RRT = \Delta\mu_X / R \cdot T$$

should hold. Under most of the chromatographic system, T is constant. Therefore, $1/R \cdot T$ will also be constant.

$$\therefore \log RRT \propto \Delta\mu_X \tag{3}$$

From the aforementioned aspect, formula [1] can be rewritten as follows :

$$\Delta\mu_B = \Delta\mu_A + \Delta\mu_X \tag{4}$$

This formula [4] can be expanded to cover

$$\Delta\mu_{DG} = \Delta\mu_{FA_1} + \Delta\mu_{FA_2} \tag{5}$$

where DG is the diacylglycerolipid and FA_1 and FA_2 are the fatty acid residues of DG. From formulae [3], [4] and [5],

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$$\log \ RRT_{DG} = \log \ RRT_{FA_1} + \log \ RRT_{FA_2} \qquad [6]$$

can be obtained. So by calculating the relative retention potential of the individual fatty acid residue, it is possible to predict the relative retention value ($=RRT$) of the individual molecular species of DG.

This idea was demonstrated by comparing the theoretically determined relative retention potential of the individual molecular species of DG and also the empirically determined one.

Methods

The RRT data of acetyldiglyceride[2,3], obtained by acetylation subsequent to phospholipase C hydrolysis of soybean phosphatidylcholine (PC), egg yolk PC, and PC from five kinds of fish muscle were reviewed. The relative retention potential of each fatty acid residue of the acetyldiglyceride, i.e., the DG, was calculated as follows:

From the identified monoacid DG molecular species,

$$\log \ RRT_{FA} = (\log \ RRT_{DG})/2 \qquad [7]$$

and from the identified diacid DG molecular species,

$$\log \ RRT_{FA_1} = \log \ RRT_{DG} - \log \ RRT_{FA_2} \qquad [8]$$
$$\text{(Unknown)} \qquad\qquad \text{(Known)}$$

or,

$$\log \ RRT_{FA_2} = \log \ RRT_{DG} - \log \ RRT_{FA_1} \qquad [9]$$
$$\text{(Unknown)} \qquad\qquad \text{(Known)}$$

The theoretically determined relative retention potential of the individual DG molecular species that denotes $\log \ RRT_{DG} = \log \ RRT_{FA_1} + \log \ RRT_{FA_2}$ (each term is calculated from formulae [7], [8] and [9]) was compared with the empirically determined one.

The "Q" value examination proposed by Dean and Dixon[4] was employed in order to minimize the error of RRT.

Results

Table 1 shows the theoretically determined relative retention potential of each fatty acid residue of the DGs that have been reviewed[2,3]. For example, from the RRT data[2,3] of $(22:6)(22:6)$, that is, 44.1, 44.2, 44.3, 44.6, 46.2, all of these could be employed for the mean value calculation according to the "Q" value examination[4]. In this case, the mean value of RRT would become 44.6 and the relative retention potential of $22:6$ fatty acid residue was calculated as $(\log 44.6)/2 = 0.8247$ (See formula [7]). In the same way, the relative retention potential of $20:5$ fatty acid residue was calculated as $(\log 37.3)/2 = 0.7859$, therefore, the relative retention potential of $(20:5)(22:6)$ can be predicted as $0.8247 + 0.7859 = 1.611$. The actual relative retention potential of $(20:5)(22:6)$ was 1.613, as shown in Table 2. The relative error of the retention potential will be $(1.613 - 1.611)/1.611 = 0.12\%$ in case of $(20:5)(22:6)$. A retention potential of $16:0$ was calculated as follows. The mean value of the RRT of $(16:0)(20:5)$ was $92.7^{2,3}$; and, the relative retention potential of this molecular species was $\log 92.7 = 1.967$. Accordingly, from

Table 1. Relative retention potential of the fatty acid residues of acetyldiglyceride on reverse phase high performance liquid chromatography.*

Fatty acid residue	Relative retention potential	Fatty acid residue	Relative retention potential
14 : 0	1.023	16 : 2	0.8670
15 : 0	1.102	17 : 2	0.9850
16 : 0	1.181	18 : 2 ω6	0.9757
17 : 0	1.229	18 : 3 ω3	0.8580
18 : 0	1.347	20 : 4 ω6	0.9090
20 : 0	1.484	22 : 4	1.027
16 : 1 ω9	0.9985	20 : 5 ω3	0.7859
17 : 1	1.081	22 : 5	0.9261
18 : 1 ω9	1.142	22 : 6 ω3	0.8247
20 : 1	1.313		
24 : 1	1.638		

* Relative retention time of (16 : 0)(22 : 6) is regarded as 100. Equipment, Hitachi 638–50; Shodex RI monitor; column, LiChrosorb RP–18 (250×8 mm; tandem); solvent, iso-propanol/acetone/methanol/acetonitrile (1 : 1 : 3 : 4, v/v); flow, 1.5 ml/min; column temperature, ambient.

formula [8], the relative retention potential of 16 : 0 can be calculated as 1.967 − 0.7859 = 1.181. Incidentally, the relative retention potential of (16 : 0)(22 : 6) can be predicted as 1.181 + 0.8247 = 2.006 and the actual relative retention potential of (16 : 0)(22 : 6) was 2.000 (See Table 2). All of the relative retention potentials of the fatty acid residues in Table 1 were calculated in the same manner. From the calculated retention potential of the fatty acid residues shown in Table 1, the relative retention potential of the individual molecular species of *DG* was theoretically predicted and it was compared with the empirically determined one as shown in Table 2. Though there are small errors between the theoretically determined retention potential and the empirically determined one, the validity of this idea is well demonstrated.

Discussion

As it is obvious from the theory of Martin[1], formulae [1] and [2] should hold for all of the partition chromatographic systems. It then follows that formulae [6], [7], [8] and [9] should also hold for all of the partition chromatographic systems of *DG* lipid molecular species regardless of the analytical condition employed for the chromatography. By introducing the *RRT* data of the dinitrobenzoyl derivatives from PC presented by Takamura *et al.*[5] and Kito *et al.*[6], instead of the acetyl derivatives that have been discussed in this study, it was also demonstrated that the theoretically determined relative retention potential of this derivative coincides well with the empirically determined one as shown in Table 3. This shows that not only

Table 2. Comparison of the predicted and the empirically determined relative retention potential of the individual molecular species of acetyldiglyceride.*

Molecular species of acetyldiglyceride	Predicted relative retention potential	Empirically determined relative retention potential	Relative error (%)
(20:5)(20:5)	1.572	1.572	**
(20:5)(22:6)	1.611	1.613	0.12
(22:6)(22:6)	1.649	1.649	**
(18:3)(22:6)	1.683	1.688	0.30
(20:4)(20:5)	1.695	1.719	1.42
(22:5)(20:5)	1.712	1.712	**
(20:4)(22:6)	1.734	1.722	0.69
(22:5)(22:6)	1.751	1.746	0.29
(18:2)(20:5)	1.762	1.788	1.48
(16:1)(20:5)	1.784	1.784	**
(18:2)(22:6)	1.800	1.825	1.39
(14:0)(20:5)	1.809	1.809	**
(16:1)(22:6)	1.823	1.824	0.01
(18:2)(18:3)	1.834	1.848	0.76
(20:4)(22:5)	1.835	1.869	1.85
(14:0)(22:6)	1.848	1.843	0.27
(22:4)(22:6)	1.852	1.852	**
(22:5)(22:5)	1.852	1.894	2.27
(18:2)(20:4)	1.885	1.917	1.70
(15:0)(20:5)	1.888	1.891	0.16
(17:2)(20:4)	1.894	1.894	**
(18:2)(22:5)	1.906	1.900	0.05
(17:1)(22:6)	1.906	1.906	**
(16:1)(20:4)	1.908	1.918	0.52
(17:2)(22:5)	1.911	1.895	0.84
(16:1)(22:5)	1.925	1.900	1.30
(15:0)(22:6)	1.927	1.927	**
(18:1)(20:5)	1.928	1.945	0.88
(14:0)(22:5)	1.949	1.915	1.74
(18:2)(18:2)	1.951	1.981	1.54
(18:1)(22:6)	1.966	1.966	**
(16:0)(20:5)	1.967	1.967	**
(16:1)(18:2)	1.974	2.000	1.32
(18:1)(18:3)	1.999	1.999	**
(14:0)(18:2)	1.999	2.000	0.05
(16:0)(22:6)	2.006	2.000	0.29
(16:0)(18:3)	2.039	2.030	0.88
(16:0)(16:2)	2.048	2.048	**
(18:1)(20:4)	2.051	2.064	0.29
(17:0)(22:6)	2.053	2.067	0.68

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