Abstract Triacylglycerol (TAG) lipases hydrolyze ester bonds in TAG and release diacylglycerol (DAG), monoacylglycerol (MAG), and FA. We present a one-step chemical derivatization method for label-free quantification of a mixture of TAG, DAG, and MAG following lipase assay by ESI-MS. Because the ionization efficiencies of TAG, DAG, and MAG are not identical, lipase reaction products, DAG and MAG, are derivatized to TAG species by esterifying their hydroxyl groups using acyl chloride, whose acyl chain contains one less (or one more) –CH\textsubscript{2} group than that of substrate TAG. This resulted in three TAG species that were separated by 14 Da from one another and exhibited similar ion responses representing their molar amounts in the mass spectra. A good linear correlation was observed between peak intensity ratios and molar ratios in calibration curve. This method enables simultaneous quantification of TAG, DAG, and MAG in lipase assay and, in turn, allows stoichiometric determination of the concentrations of FAs released from TAG and DAG separately. By applying this strategy to measure both TAG and DAG lipolytic activities of the yeast Tgl2 lipase, we demonstrated its usefulness in studying enzymatic catalysis, as lipase enzymes often show dissimilar activities toward these lipids.—Ham, H. J., J. Seo, H.-J. Yoon, and S. K. Shin. Label-free measurement of the yeast short chain TAG lipase activity by ESI-MS after one-step esterification. J. Lipid Res. 2017. 58: 625–631.

Supplementary key words chemical derivatization • triacylglycerol • lipolysis • specific activity • electrospray ionization-mass spectrometry

Triacylglycerol (TAG), a nonpolar storage lipid, serves as the most efficient energy source in diverse cells, provides building blocks for cell membrane lipid biosynthesis, and releases second messenger for cell signaling upon degradation (1). Thus, TAG mobilization plays a key role in energy homeostasis and cell proliferation in the biological systems. Lipolytic enzymes, lipases, are involved in TAG catabolism and deregulation of these activities perturbs cell physiology, leading to metabolic disorders and pathological conditions in many organisms.

Lipases carry hydrolyzing activities toward TAG and diacylglycerol (DAG), forming DAG plus FA and monoacylglycerol (MAG) plus FA, respectively. Their catalytic activities to TAG and DAG can be very different, though. Because lipase assay produces a mixture of TAG, DAG, MAG, and FA, simultaneous quantification of these lipids is the first step in enzymatic kinetics. Traditional methods for the determination of lipolytic activity include pH titrimetry and TLC analysis (2, 3). The TLC-based method requires radioactive- or fluorescence-labeled substrates/products for detection and monitors the disappearance of substrates and/or the appearance of products on TLC plate. Either autoradiographic or densitometric analysis can be carried out for quantification. The titration method is an indirect assay, measuring the amount of base added to neutralize the released FA. The latter method is restricted to a specific pH range and less sensitive than the former one. While lipase activities are determined by the amount of FAs released from substrates per minute under standard condition, the methods targeting FAs are unable to distinguish hydrolyzing activities toward TAG and DAG.

More recently, MS linked to LC has been widely used in the field of lipid research (4–6). In particular, ESI-MS has greatly facilitated the quantitative and sensitive analysis of both polar and neutral lipids since the first reports of phospholipid analysis (7, 8). For instance, an LC-ESI-MS system has been applied to determine lipase activity by monitoring the FA production (9). Further progress in lipid research has been made by combining chemical derivatization with high-resolution nano ESI-MS in lipidome analysis (10).
TAG, DAG, and MAG are all uncharged fatty acyl ester derivatives of glycerol. In ESI-MS, this class of neutral lipids has been routinely analyzed as metal adduct ions after introducing charge states with alkali metals (11, 12). For analysis of DAG species, a derivatization method using N-chloro-3-bromobenzamide (NCBBA) has significantly improved the quantification efficiency of ESI-MS (13, 14). By taking a MS-based shotgun lipidomics approach, the latter method allows accurate and reproducible analysis of all of the cellular DAG species, including 1, 2-, and 1,3-DAG isomers (14). However, TAG, DAG, and MAG carrying the same acyl side chain(s) show very dissimilar ion responses and, in turn, different peak intensities, making their simultaneous quantification difficult. Even within each species of a lipid class, detection sensitivity of ESI-MS varies significantly depending on the acyl chain length and the unsaturation index in addition to the experimental setups.

Via simple esterification, here we report a strategy to obtain identical ion responses from TAG, DAG, and MAG that contain the same acyl side chain(s). We used ESI-MS to quantify a mixture of TAG, DAG, and MAG following chemical derivatization and investigated quantification linearity and detection sensitivity over a wide dynamic range. Biological application of this method was demonstrated by measuring lipolytic activities of the yeast Saccharomyces cerevisiae Tgl2 protein.

MATERIALS AND METHODS

Materials

Acyl chlorides (heptanoyl and nonanoyl chlorides), N-bromosuccinimide (NBS), 1-octanoyl-3-octanoyl-3-(N,N-dimethylglycine) (BNBDA), N,N-dimethylglycine has significantly improved the quantification efficiency of ESI-MS (13, 14). By taking a MS-based shotgun lipidomics approach, the latter method allows accurate and reproducible analysis of all of the cellular DAG species, including 1, 2-, and 1,3-DAG isomers (14). However, TAG, DAG, and MAG carrying the same acyl side chain(s) show very dissimilar ion responses and, in turn, different peak intensities, making their simultaneous quantification difficult. Even within each species of a lipid class, detection sensitivity of ESI-MS varies significantly depending on the acyl chain length and the unsaturation index in addition to the experimental setups.

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Preparation of Tgl2 lipase

To purify hemagglutinin (HA)-tagged Tgl2 protein, yeast cells (strain YH058d2; 1g22) harboring the plasmid pYNO4-HA-TGL2 were cultured as described previously (16). Yeast lysates (~700 µl) prepared from ~4 × 10^9 cells were incubated with 50 µl of anti-HA affinity matrix (clone 3F10, Roche) at 4°C overnight. The beads were washed extensively, resuspended with 200 µl of 20 mM sodium phosphate buffer (pH 7.4), and used for lipase assay. About 1/10 of HA-Tgl2 protein bound to the anti-HA affinity beads was analyzed by SDS-PAGE followed by SYPRO Ruby staining (Molecular Probes, Eugene, OR) and quantified using the fluorescence gel imaging system (VersaDoc 5000 MP, Bio-Rad). The amount of Tgl2 protein was determined from the calibration curve using BSA as an internal standard.

Lipase assay and chemical esterification

Lipase assay was performed by the published method (16). Briefly, C8:0/C8:0/C8:0 TAG was added to the assay buffer composed of 150 µl of 50 mM Tris-HCl (pH 8.0) and 20 µl of a BSA solution (20 mg/ml) to a final concentration of 1.74 mM (296 nmol). The mixture was sonicated at 37°C until the solution became cloudy (~4 min) and then incubated at 37°C in the presence of 50 µl of 200 mM MgCl_2 (total volume, 220 µl). The lipase reaction was initiated by adding 200 µl of HA-Tgl2 protein (4 µg) purified as above. Aliquots (20 µl each) were taken out at the start (time 0) and end (time 60 min) of incubation at 37°C and immediately analyzed by TLC to monitor the progress of the lipase reaction. Lipid species in the remaining reaction mixture (266 nmol; 380 µl of 0.7 mM) were extracted by adding 1 ml of chloroform and dried under N₂ flow for chemical esterification. The residues were resuspended in DCM, esterified, and prepared for ESI-MS analysis as described above.

Experimental setup and data analysis

The ESI mass spectra were obtained by using an ESI triple quadrupole mass spectrometer (AB SCIEX 2000 QTRAP; Forster City, CA). Sample solutions were directly transferred to the electrospray tip by using a syringe pump at a flow rate of 10 µl/min.
and electrosprayed with a N2 nebulizer gas. The electrospray voltage was 5 kV. Lipid-metal ion adducts were collected in the ion trap for 200 ms and scanned for 1.6 s to obtain a single scan spectrum in the m/z range 200–800. After 200 ms of dead time, ion collection was started again in the trap. A single scan spectrum was obtained every 2 s. The final ESI mass spectrum was constructed by summing total 30 single-scan spectra obtained for 1 min. All data were acquired in the positive ion mode.

RESULTS AND DISCUSSION

Quantification concept

In ESI-MS, the ion response of lipid highly depends on the lipid structure and the mass range. TAG, DAG, and MAG containing the same acyl side chain(s) yield very different ion intensities due to their nonidentical ionization efficiencies. For instance, the ESI mass spectrum of a 1:1:1 mixture of TAG (C8:0/C8:0/C8:0), DAG (C8:0/C8:0), and MAG (C8:0) presents three major ions of [TAG + Na]+, [DAG + Na]+, and [MAG + Na]+ at m/z 494.2, 368.1, and 241.9, respectively (supplemental Fig. S1). The lipids were dissolved with NaCl in methanol to promote the formation of sodium ion adducts. As shown in supplemental Fig. S1, the peak intensities of [TAG + Na]+, [DAG + Na]+, and [MAG + Na]+ are not identical, although each lipid was mixed at equimolar concentration.

Relative quantification of lipid species by ESI-MS requires similar ion responses representing their molar amounts. While the ionization efficiency is mainly determined by the lipid structure and the molecular weight, the same class of lipid species with one or two different carbon number(s) gives almost identical ion response. We thus esterified DAG and MAG to TAG species containing one and two less (or more) –CH2 group(s) than unmodified DAG and MAG, respectively, by using acyl chloride (supplemental Fig. S2). NBS was added to the reaction mixture to remove acidic byproduct and facilitate the esterification. The resulting TAG derived from DAG is named to TAG′ and the mass difference between TAG and TAG′ is 14 Da. Similarly, TAG″ is derived from MAG and the mass difference between TAG and TAG″ is 28 Da. When DAG or MAG whose carbon number in the acyl group is 8, for instance, is modified to TAG′ or TAG″ by using heptanoyl chloride (C7), whose carbon number is 7, a 2:1:2 mixture of TAG, TAG′, and TAG″ exhibits 2:1:2 peaks of sodium ion adducts at m/z 494.2, 508.1, and 522.2, respectively (supplemental Fig. S1C). Important, the peak intensity ratio faithfully reproduced the pre-mixed molar ratio. Chemical esterification using nonanoyl chloride (C9), whose carbon number is 9, resulted in ion peaks of TAG, TAG′, and TAG″ at m/z 494.2, 508.1, and 522.2, respectively (supplemental Fig. S1D). These data suggest that our strategy minimizes the effects of lipid structure on ion response and enables simultaneous quantification of TAG, DAG, and MAG after lipase assay by using ESI-MS. Alternatively, the esterification can be performed by using stable isotope-labeled acyl chloride. Octanoyl chloride-d3 [CD3(CH2)6COCl], for instance, can convert

Fig. 1. Plots of the relative signal intensity ratios, [TAG′]/[TAG] and [TAG″]/[TAG], as a function of reaction time. Esterification using C7 (A); esterification using C9 (B). The temporal data for both [TAG′]/[TAG] and [TAG″]/[TAG] were fitted to the pseudo first-order rate equations for DAG-to-TAG′ and MAG-to-TAG″ esterifications in excess of acyl chloride, respectively.

Fig. 2. Linearity plots for the quantification of C8:0/C8:0 DAG and C8:0 MAG by ESI-MS. For both DAG → TAG′ (A) and MAG → TAG″ (B) reactions, C7 or C9 was added to the sample mixtures S1–S4 (Table 1).
DAG (C8:0/C8:0) and MAG (C8:0) to TAG-Δ3 (C8:0/C8:0/C8:0) and TAG-Δ6 (C8:0/C8:0/C8:0), respectively. Although this almost completely eliminates the structural effects on ion response, we prefer the use of label-free C7 or C9 because it is cost-effective and produces a mass difference of 14 Da (3 Da in the case of octanoyl chloride-Δ3) between TAG, TAG′, and TAG″. Ion peaks of these TAG species are well-separated in the mass spectra.

Esterification kinetics

The progress of the esterification reaction was checked by monitoring the relative ratios of TAG′ over TAG and TAG″ over TAG ([TAG′]/[TAG] and [TAG″]/[TAG]) in the ESI mass spectra as a function of reaction time (Fig. 1). When the lipid mixture was treated with C7, the measured ratios of [TAG′]/[TAG] and [TAG″]/[TAG] were 1.00 ± 0.19 and 0.97 ± 0.20 at 7 h, respectively (Fig. 1A). Thus, reaction yields for DAG and MAG esterifications were 100% and 97%, respectively. Both reactions were nearly completed at 2 h. In the case of incubation with C9, the measured ratios of [TAG′]/[TAG] and [TAG″]/[TAG] were 0.84 ± 0.11 and 0.70 ± 0.10 at 7 h, respectively, resulting in reaction yields of ~84% and ~70%, respectively (Fig. 1B). In this case, both DAG → TAG′ and MAG → TAG″ reactions plateaued after 4 h.

The observed rate constant (k_{obs}) for the appearance of TAG′ from DAG-to-TAG′ esterification was 3.83 h⁻¹ using C7 (Fig. 1A) and 1.32 h⁻¹ using C9 (Fig. 1B). Hence, the bimolecular rate constant for the reaction of DAG with acyl chloride was 8.25 cm³ mol⁻¹ s⁻¹ using C7 and 4.55 cm³ mol⁻¹ s⁻¹ using C9. Interestingly, the MAG-to-DAG′ esterification proceeded faster than the DAG-to-TAG′ esterification in both cases using C7 and C9. This is mainly because the hydroxyl group in DAG is more sterically hindered than those in MAG, indicating that the steric hindrance plays a significant role in the kinetics of esterification.

It seems that chemical esterification using C7 resulted in higher yield and faster rate than that using C9 due to the shorter alkyl chain of C7. Our observation is in accord with the previous report on the esterification of tertiary alcohols in steroids using acyl chloride, exhibiting the reaction yield of 85–95% at the reaction time of 10–12 h (15). Both studies suggested that the reaction yield was higher and the reaction rate was faster, when the aliphatic chain length of acyl chloride was shorter. The kinetics of esterification can also be influenced by other parameters, such as catalyst, pH, solvent, temperature, and so on.

Quantification linearity and detection limit

We then examined the relationship between the measured ratios of TAG, TAG′, and TAG″ peak intensities and the predetermined molar ratios of TAG, DAG, and MAG to account in the equation [k_{obs} / (3,600 s⁻¹)]/[acyl chloride]]. In the case of consecutive MAG-to-TAG″ esterifications, the rate constant for the appearance of TAG″ from DAG′-to-TAG″ esterification can be assumed to be identical to that for the appearance of TAG′ from DAG-to-TAG′ esterification. Then the observed rate constant for the appearance of DAG′ from MAG-to-DAG′ esterification was 6.76 h⁻¹ using C7 (Fig. 1A) and 1.80 h⁻¹ using C9 (Fig. 1B), which resulted in the bimolecular rate constant of 14.56 cm³ mol⁻¹ s⁻¹ using C7 and 4.55 cm³ mol⁻¹ s⁻¹ using C9.
assess their simultaneous quantification after chemical esterification. The lipid samples (C8:0/C8:0/C8:0 TAG, C8:0/C8:0 DAG, and C8:0 MAG) were premixed in DCM at four different molar ratios (S1, 2:1:2; S2, 4:1:2; S3, 3:1:1; and S4, 8:1:2, respectively, of [TAG]:[DAG]:[MAG]) prior to derivatization. The sample mixtures S1–S4 were esterified using C7 or C9, and subsequently analyzed by ESI-MS. The relative amounts of TAG, DAG, and MAG were determined using C7 or C9, and subsequently analyzed by ESI-MS. The sample mixtures S1–S4 were esterified using C7 or C9, and subsequently analyzed by ESI-MS. The relative amounts of TAG, DAG, and MAG were determined by measuring the peak heights of sodium ion adducts, [TAG + Na]+ at m/z 494.2, [TAG ′ + Na]+ at m/z 480.1/508.1, and [TAG ″ + Na]+ at m/z 466.1/522.2, respectively. We plotted the measured ratios of [TAG]/[DAG] and [TAG]/[MAG], respectively (Fig. 2). Before or after dilution, the mole fraction ratios of TAG:TAG ′:TAG ″ are 0.59:0.18:0.24 and 0.62:0.15:0.23, as expected from the 10:3:4 and 8:2:3 premixed molar ratios of [TAG]:[DAG]:[MAG], respectively. Five-fold serial dilutions of each master solution were analyzed by ESI-MS (Tables 3, 4). Before or after dilution, the mole fraction ratios of TAG:TAG ′:TAG ″ are 0.59:0.18:0.24 and 0.62:0.15:0.23, as expected from the 10:3:4 and 8:2:3 premixed molar ratios of [TAG]:[DAG]:[MAG], respectively. After esterification using C7 (Table 3), the observed mole fractions determined by measuring signal intensities of TAG, TAG ′, and TAG ″ in the mass spectra were in good agreement with the expected ones (TAG:TAG ′:TAG ″ = 0.59:0.18:0.24) up to a mixture of 0.40 pmol of TAG, 0.12 pmol of TAG ′, and 0.16 pmol of TAG ″ and the signal-to-noise ratio was greater than 2. In the case of esterification using C9 (Table 4), the expected mole fraction ratio (TAG:TAG ′:TAG ″ = 0.62:0.15:0.23) was maintained up to a mixture of 16 fmol of TAG, 4 fmol of TAG ′, and 6 fmol of TAG ″ and the signal-to-noise ratio was greater than 3. Previously, a detection limit of 0.1 pmol was achieved for TAG by using ESI-MS (12). Although the detection sensitivity is highly dependent on the instrumental conditions, our results obtained from the mixtures of TAG species by using an ESI triple quadrupole mass spectrometer are comparable to the results reported by others (12). A further increase in the detection sensitivity could be achieved by adopting higher performance instruments.

**MS-based measurement of the Tgl2 lipase activity**

We applied our esterification strategy to study lipolytic catalysis of the yeast Tgl2 protein. In the yeast *Saccharomyces cerevisiae*, TAG is mobilized by three lipases, Tgl3–5, present

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**TABLE 3. Summary of the expected concentrations of TAG, TAG ′, and TAG ″ and their mole fractions observed in the mass spectra**

| Expected Concentration | Observed Mole Fraction* |
|------------------------|-------------------------|
| [TAG]                  | [TAG ′]                 | [TAG ″] |
| 1 50 µM (50 pmol)      | 15 µM (15 pmol)         | 20 µM (20 pmol) |
| 2 10 µM (10 pmol)      | 3.0 µM (3.0 pmol)       | 4.0 µM (4.0 pmol) |
| 3 2 µM (2.0 pmol)      | 0.60 µM (0.60 pmol)     | 0.80 µM (0.80 pmol) |
| 4 0.40 µM (0.40 pmol)  | 0.12 µM (0.12 pmol)     | 0.16 µM (0.16 pmol) |
| 5 80 nM (80 fmol)      | 24 nM (24 fmol)         | 32 nM (32 fmol) |
| 6 16 nM (16 fmol)      | 4.8 nM (4.8 fmol)       | 6.4 nM (6.4 fmol) |
| 7 3.2 nM (3.2 fmol)    | 1.0 µM (1.0 fmol)       | 1.5 nM (1.3 fmol) |

Five-fold serial dilutions were prepared from a 10:3:4 mixture of TAG, DAG, and MAG after esterification using C7. The mole fraction ratio of TAG:TAG ′:TAG ″ is expected to be 0.59:0.18:0.24.

*Errors include an instrumental response error (5%).

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**TABLE 2. Summary of the mole fractions of TAG, DAG (or TAG ′), and MAG (or TAG ″)**

| Predetermined Mole Fraction | C7 | C9 |
|-----------------------------|----|----|
| TAG            | DAG | MAG |
| TAG            | TAG ′| TAG ″|
| TAG            | TAG ′| TAG ″|

| S1 | S2 | S3 | S4 |
|----|----|----|----|
| 0.40| 0.20| 0.40| 0.40|
| 0.57| 0.14| 0.29| 0.29|
| 0.60| 0.20| 0.29| 0.29|
| 0.73| 0.09| 0.18| 0.18|

*Errors include an instrumental response error (5%).
in the lipid droplets and the Tgl2 lipase located in the mitochondria (16–19). Among these lipases, Tgl2 is highly reactive toward both C8:0/C8:0/C8:0 TAG and C8:0/C8:0 DAG (16). We purified Tgl2 protein from the yeast whole cell lysates and performed a lipase assay using C8:0/C8:0/C8:0 TAG (glyceryl trioctanoate) as substrate. Before ESI-MS analysis, esterification of assay products was carried out by using C7 and C9, as described in the Materials and Methods. The amount of TAG, DAG, or MAG following the Tgl2 lipase assay was determined from the initial concentration of TAG (0.7 mM) after measuring the relative abundances of TAG′ and TAG″ in the mass spectra (Fig. 4). Reaction yields for DAG-to-TAG′ and MAG-to-TAG″ esterifications using C7 and C9 were taken into account in the conversion of the relative abundances of TAG′ and TAG″ to the concentrations of DAG and MAG, respectively. The TAG′ and TAG″ production yields of 100% and 97% in the case of C7 (Fig. 1A) or those of 84% and 70% in the case of C9 (Fig. 1B), respectively, were reflected in the calculated concentrations of DAG and MAG (Table 5).

Next, we calculated the amounts of FAs liberated from TAG and DAG to measure specific activities of Tgl2 lipase toward TAG and DAG, respectively. Because the stoichiometric amount of FA derived from TAG is the sum of [DAG] × 1 and [MAG] × 2, as listed in Table 5, the specific activity of Tgl2 toward TAG can be obtained by using the equation 

\[ \frac{\text{[FA]}_{\text{from TAG}}}{\text{initial concentration of TAG at the start of lipase assay}} \times \text{reaction time} \times \text{volume (380 µl)} \times \text{[FA]}_{\text{from TAG}}/ \]

\[ k_1 \] and \[ k_2 \] are the rate constants. Because we know the initial concentration of TAG and the final concentrations of DAG, and MAG after 60 min lipolysis (Table 5), we can calculate the rate constants \[ k_1 \] and \[ k_2 \] from the following equations for consecutive reactions (20):

\[ \frac{\text{[TAG]}_{0}}{\text{[TAG]}_{0} - \text{[TAG]}_{t}} \times \exp (\frac{\text{[DAG]}}{1}) \times \exp (\frac{\text{[MAG]}}{2}) \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]

\[ \text{[FA]}_{\text{from DAG}} = \frac{\text{[FA]}_{\text{from DAG}}}{\text{[FA]}_{\text{from DAG}} + \text{[FA]}_{\text{from DAG}}} \]
from DAG is \([MAG] \times 1\), the specific activity of Tgl2 toward DAG can be calculated by using the equation \((([[DAG]_0 - [DAG]]) \times \text{volume (380 \(\mu\text{l}\))} / ([\text{enzyme amount (4 \(\mu\text{g}\))} \times \text{reaction time (60 min)})] \times [FA]_{\text{from DAG}} / [MAG])\). The specific activity toward DAG was 1.06 ± 0.05 \(\mu\text{mol min}^{-1} \text{ mg}^{-1}\) using C7 and 0.99 ± 0.06 \(\mu\text{mol min}^{-1} \text{ mg}^{-1}\) using C9 (Table 6). The specific lipase activity of Tgl2 toward DAG was 1.5–1.6 times higher than that toward TAG, showing a good agreement with the previous result (16). Therefore, we have successfully measured both TAG and DAG hydrolyzing activities of the yeast Tgl2 lipase and demonstrated the feasibility of this method for biological samples.

In conclusion, we describe a method enabling simultaneous quantification of TAG, DAG, and MAG after lipase assay without the aid of radioactive or fluorescent labeling. This method is based on a quantification strategy combining chemical esterification and ESI-MS and provides quantitative data for all products of lipase assay, including FA. Resulting information will allow the determination of lipolytic activities in the biological systems.

TABLE 6. Specific enzyme activity of the yeast Tgl2 lipase

| Lipids | C7                  | C9                  |
|-------|---------------------|---------------------|
| TAG   | 0.71 ± 0.03         | 0.62 ± 0.03         |
| DAG   | 1.06 ± 0.05         | 0.99 ± 0.06         |

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