Journal of Environmental Biotechnology
Vol. 17, No. 1, 69–72, 2017

Technical Note

Application of Bio-Based Organic Acid, D-Glyceric Acid: Synthesis and Interfacial Property of Dihexanoyl Glycerate

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(Received: 22 December, 2016/Accepted: 11 January, 2017)

Key words: biorefinery, green surfactant, glycerol, glyceric acid, diacyl glycerate

Glycerol is produced as a by-product in the growing biodiesel and oleochemical industries, and many projects are under way to convert glycerol into various marketable and value-added products [11,18]. Glyceric acid (2,3-dihydroxypropanoic acid; GA) is a natural minor organic compound in specific plants [10,12], but can be mass-produced biotechnologically from glycerol [3,6,7,16]. One of the significant advantages of producing GA from glycerol using acetic acid bacteria is that *Acetobacter tropicalis* provides a chiral skeleton in the C-2 of GA and produces enantiopure D-GA with 99% enantiomer excess (ee) [7]. Therefore, applications of D-GA and its derivatives should be developed to expand its commercial production and application.

Biological activities of D-GA and those of GA derivatives, such as diacyl GA and glucosyl GA have been reported [1,5,8,9,13]. In 2001, Lesová et al. reported that glyceric acids esterified with long acyl chains (>C16) exhibit antitrypsin activity [9], however, their hydrophobic nature, derived from the long acyl chains, limits the number of studies that can be performed on their physical and biological properties. For example, we previously synthesized diacyl GAs with acyl chain lengths of C16 and C18 and investigated their biological properties [4,14,15], but the trypsin inhibitory efficiency of dioleoyl GA was low, likely due to its low water solubility [4].

To improve water solubility of diacyl GAs, we recently synthesized a diacyl GA with a shorter octanoyl group (C8) acyl chain and investigated its physical properties [17]. Synthesized dioctanoyl GA (diC8GA) was not soluble in water, whereas its sodium salt (diC8GA-Na) was water soluble and had surface tension-lowering properties; the critical micelle concentration (CMC) was 0.82 mM, and surface tension at the CMC was 25.5 mN/m [17]. These data support the usefulness of diacyl glycerates as new surfactants; however, water solubility and interfacial properties of diacyl glycerates with acyl chains other than C8 have not been investigated. In this study, we synthesized more water-soluble diacyl GA sodium salts with a shorter hexanoyl group (C6) acyl chain than that of diC8GA-Na (Fig. 1) and investigated their surface-tension-lowering property.

D-GA with an ee of 99% was prepared from glycerol by oxidative fermentation using *Acetobacter tropicalis* NBRC16470 [3,7]. The D-GA calcium salts were converted to free acids using DOWEX cation exchange resin (Dow Chemicals, Midland, MI, USA), as described previously [2]. Hexanoyl chloride were purchased from Wako Pure Chemicals (Osaka, Japan). D-GA was acylated in anhydrous acetone in

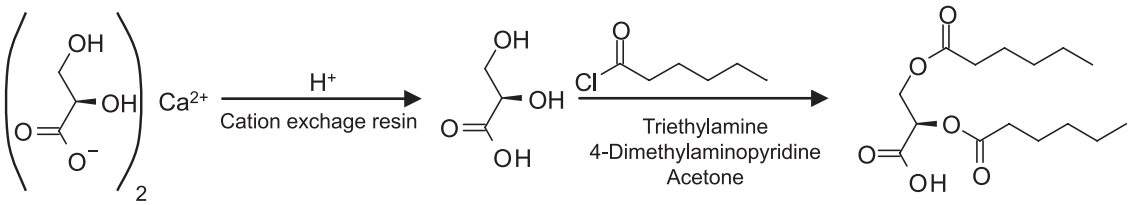

Fig. 1. Synthesis of dihexanoyl glyceric acid (diC6GA).

the presence of dimethylaminopyridine and triethylamine, as described for diC8GA [17]. A 3.04-mL aliquot of D-GA solution (10 mmol in acetone) was added to a 100-mL three-neck round-bottom flask and maintained at 0°C on ice to synthesize diC6GA. Anhydrous acetone (35.7 mL), triethylamine (3.04 mL, 22 mmol), and dimethylaminopyridine (87.3 mg, 0.7 mmol) were added to the solution with stirring, and the flask was purged with dry air. Acylation was started by drop-wise addition of hexanoyl chloride (1.35 mL, 33 mmol) over 30 min at 0°C. The solution was incubated at 0°C for an additional 30 min. Reaction progress was monitored by thin layer chromatography (TLC). TLC was developed with chloroform : methanol (8 : 2), and the organic compounds were visualized by heating at 120°C for 5 min with a 5% (w/v) phosphoric-molybdate solution in ethanol containing 5% (v/v) sulfuric acid and 0.6% phosphoric acid. After the spot corresponding to GA disappeared, the reaction mixture was filtered with no. 40 filter paper (GE Healthcare UK Ltd., Little Chalfont, UK), and the filtrates were evaporated *in vacuo*. To the resulting material, 1 M HCl and ethyl acetate (33 mL) were added, and the organic layer was collected. The fraction was dried using anhydrous Na_2SO_4, and the solvents were removed by evaporation. The crude oil produced was purified using silica gel chromatography with hexane, hexane : ethyl acetate (8 : 2, v/v), and ethyl acetate. Fractions containing diC6GA were combined, dried using anhydrous Na_2SO_4, and concentrated *in vacuo*. The purified diC6GA was obtained with a yield of 58.3%.

The resulting compound was characterized by nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS). The ^1H and ^{13}C NMR spectra in $CDCl_3$ were recorded with a Bruker AV-400 NMR spectrometer (Bruker, Karlsruhe, Germany) at 27°C. ^1H and ^{13}C chemical shifts δ (ppm) were: 5.4 (t, 6H), 4.5 (m, 2H), 2.4 (m, 4H), 1.6 (quin, 4H), 1.3 (br, 12H), and 0.88 (t, 6H) for ^1H-NMR (Fig. 2A), and 173.31, 172.90 and 171.59 (C=O), 69.72 (C2), 62.26 (C3), 33.8 (α-C), 24.33 (β-C), 22.27 (-CH_2-), and 13.87 (-CH_3) for ^{13}C-NMR (Fig. 2B). The ^1H NMR spectrum showed diacylation of the hydroxyl groups in GA, whereas the ^{13}C NMR spectrum showed the presence of three types of carbonyl groups. Collectively, these data confirmed synthesis of diC6GA.

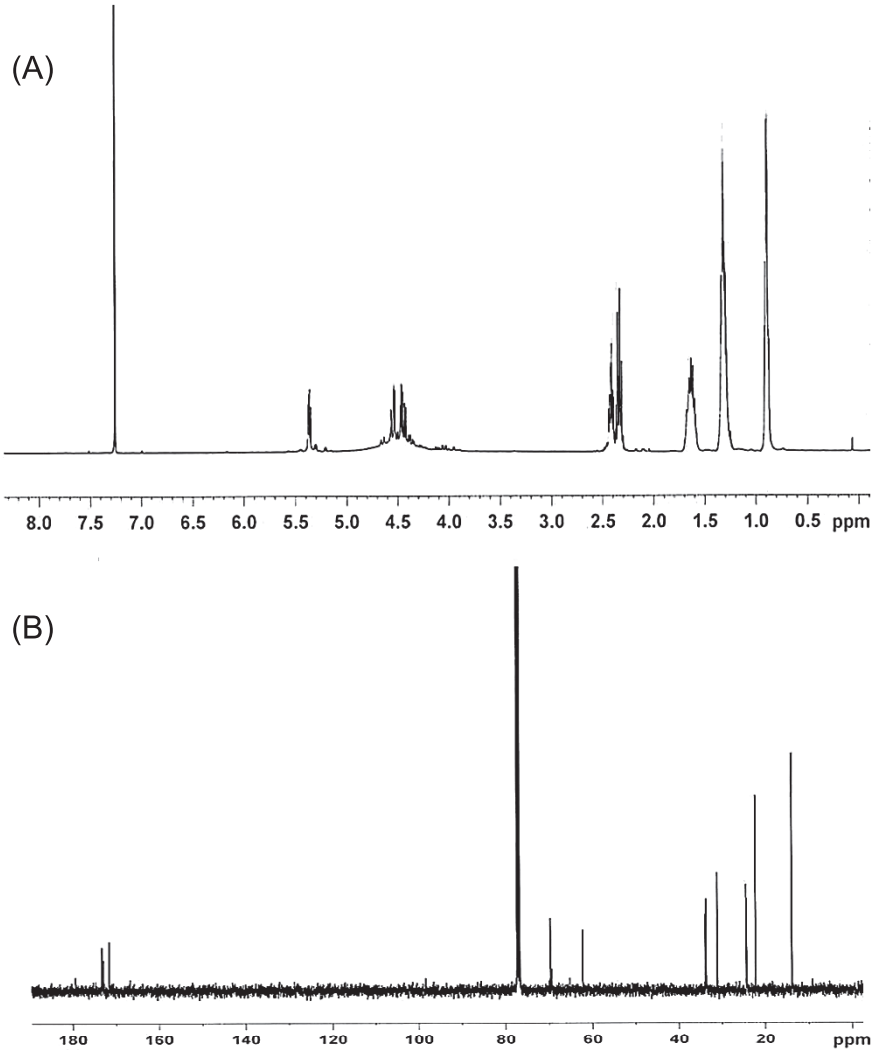

Fig. 2. (A) 400 MHz ^1H and (B) 100 MHz ^{13}C NMR spectra of dihexanoyl glyceric acid (diC6GA) in $CDCl_3$.

LC-MS was performed on a Shimadzu LC-MS 2020 system (Shimadzu, Otsu, Japan) equipped with a reverse-phase Synergi 4-μm column (150×2.0 mm, Phenomenex, Torrance, CA, USA). Samples were eluted in 0.1% (v/v) formic acid : acetonitrile (15 : 85, v/v) at 0.2 mL/min, and the column was kept at 40°C during analysis. Effluents were ionized by electrospray-ionization and detected in negative ion mode with an *m/z* range of 50–2,000. The synthesized diC6GA was dissolved in a methanol : water (1 : 1) solution and converted to a sodium salt by neutralization with an equivalent volume of 1 M NaOH. The total ion current chromatogram (TIC) from LC-MS revealed that diC6GA was eluted at approximately 2.9 min, and $[M-H]^-$ ($m/z=301$) and $[2M-H]^-$ ($m/z=603$) were the main ion forms with additional adduct ions (formate and proton adducts). TIC traces showed a dominant peak at 2.9 min with no other peaks, indicating the high purity of the diC6GA.

The diC6GA dissolved in methanol was converted to its sodium salt by titration with NaOHaq [17]. After the methanol was evaporated, the resulting diC6GA-Na was recovered by lyophilization. Molecular weights of diC6GA and diC6GA-Na were calculated from their chemical structures, and then the hydrophilic-lipophilic balance (HLB) values were derived directly from the molecular structures according to Griffin's expression equation: HLB=20×[1–(mass of hydrophobic part/total molecular mass)]. The data for HLB of diC6GA and diC6GA-Na calculated from their chemical structures were 10.59 and 11.17, respectively.

As diC6GA-Na was water-soluble and the resulting aqueous 10 mM solution as well as the 200 mM solution were clear, the surface tension of the aqueous solution containing diC6GA-Na was measured at 25°C using the pendant drop method with an automatic interfacial tensiometer (DM500, Kyowa Interface Science, Niiza, Japan) and Drop Shape Analysis software (FAMAS v2.01, Kyowa Interface Science). The CMC of the compound was calculated from the cross-point on the surface tension curve. The surface tension value at CMC (γCMC) was also determined. As shown in Figure 3, a decrease in the surface tension of diC6GA-Na was observed with increasing concentration. The CMC of diC6GA-Na was calculated as 2.92 mM from the cross-point of the surface tension plot. Generally, solubilization in water increases as chain length of the acyl group becomes shorter; therefore, the CMC value of diC6GA-Na was higher than that of diC8GA-Na (0.82 mM) [17]. The γCMC value was 33.9 mN/m, although that of diC8GA-Na was 25.5 mN/m [17]. This result shows the potential of diC6GA-Na to be a new green surfactant.

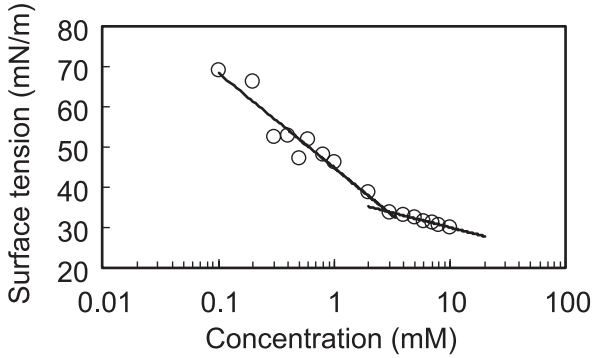

Fig. 3. Surface tension-concentration plot for dihexanoyl glyceric acid sodium salt (diC6GA-Na).

In summary, we synthesized dihexanoyl GA sodium salt from GA and hexanoyl chloride for the first time. The values of CMC and γCMC of diC6GA-Na were 2.92 mM and 33.9 mN/m, respectively. Considering that the CMC value of diC6GA (2.92 mM) is lower than that of commercially available synthetic surfactant, sodium dodecyl sulfate (SDS; CMC, 8.1 mM) [2], we can decrease the amount of surfactant for use in surface-active applications. Also, diC6GA-Na exhibited superior surface-tension-lowering activity at CMC (33.9 mN/m) compared to that of SDS (γCMC, ca. 38 mN/m) [2]. Although the previously synthesized diC8GA-Na had superior surface-tension-lowering properties in water compared to those of diC6GA-Na, development of some applications suitable for diC6GA-Na are now underway in order to make use of the advantages of diC6GA-Na such as higher water solubility.

Acknowledgments

This study was funded, in part, by KAKENHI No. 25850064.

References

1) Eriksson, C.J.P., T.P.S. Saarenmaa, I.L. Bykov, and P.U. Heino. 2007. Acceleration of ethanol and acetaldehyde oxidation by D-glycerate in rats. Metabolism. 56: 895–898.
2) Fukuoka, T., S. Ikeda, H. Habe, S. Sato, H. Sakai, M. Abe, D. Kitamoto, and K. Sakaki. 2012. Synthesis and interfacial properties of monoacyl glyceric acid as a new class of green surfactants. J. Oleo Sci. 61: 343–348.
3) Habe, H., T. Fukuoka, D. Kitamoto, and K. Sakaki. 2009. Biotransformation of glycerol to D-glyceric acid by *Acetobacter tropicalis*. Appl. Microbiol. Biotechnol. 81: 1033–1039.
4) Habe, H., T. Fukuoka, S. Sato, D. Kitamoto, and K. Sakaki. 2011. Synthesis and evaluation of dioleoyl glyceric acids showing antitrypsin activity. J. Oleo Sci. 60: 327–331.
5) Habe, H., S. Sato, T. Fukuoka, D. Kitamoto, and K. Sakaki. 2011. Effect of glyceric acid calcium salt on the viability of ethanol-dosed gastric cells. J. Oleo Sci. 60: 585–590.
6) Habe, H., Y. Shimada, T. Fukuoka, D. Kitamoto, M. Itagaki, K. Watanabe, H. Yanagishita, and K. Sakaki. 2009. Production of glyceric acid by *Gluconobacter* sp. NBRC3259 using raw glycerol. Biosci. Biotechnol. Biochem. 73: 1799–1805.
7) Habe, H., Y. Shimada, T. Yakushi, H. Hattori, Y. Ano, T. Fukuoka, D. Kitamoto, M. Itagaki, K. Watanabe, H. Yanagishita, K. Matsushita, and K. Sakaki. 2009. Microbial production of glyceric acid, an organic acid that can be mass produced from glycerol. Appl. Environ. Microbiol. 75: 7760–7766.
8) Handa, S.S., A. Sharma, and K.K. Chakraborti. 1986. Natural products and plants as liver protecting drugs. Fitoterapia. 57: 307–351.
9) Lesová, K., M. Sturdíková, B. Proksa, M. Pigos, and T. Liptaj. 2001. OR-1—a mixture of esters of glyceric acid produced

by *Penicillium funiculosum* and its antitrypsin activity. Folia Microbiol. 46: 21–23.
10) Morrison, R.J., and P.C. Dekock. 1959. Glyceric acid in broad bean (*Vicia faba* L.). Nature. 184: 819.
11) Pagliaro, M., R. Ciriminna, H. Kimura, M. Rossi, and C. Della Pina. 2007. From glycerol to value-added products. Angew. Chem. Int. Ed. 46: 2–20.
12) Palmer, J.K. 1956. Occurrence of D-glyceric acid in tobacco leaves. Science. 123: 415.
13) Sato, S., D. Kitamoto, and H. Habe. 2014. In vitro evaluation of glyceric acid and its glucosyl derivative, α-glucosylglyceric acid, as cell proliferation inducers and protective solutes. Biosci. Biotechnol. Biochem. 78: 1183–1186.
14) Sato, S., H. Habe, T. Fukuoka, D. Kitamoto, and K. Sakaki. 2011. Synthesis of dilinoleoyl-D-glyceric acid and evaluation of its cytotoxicity to human dermal fibroblast and endothelial cells. J. Oleo Sci. 60: 483–487.
15) Sato, S., H. Habe, T. Fukuoka, D. Kitamoto, and K. Sakaki. 2012. Stepwise synthesis of 2,3-*O*-dipalmitoyl-D-glyceric acid and an in vitro evaluation of its cytotoxicity. J. Oleo Sci. 61: 337–341.
16) Sato, S., N. Morita, D. Kitamoto, T. Yakushi, K. Matsushita, and H. Habe. 2013. Change in product selectivity during the production of glyceric acid from glycerol by Gluconobacter strains in the presence of methanol. AMB Express. 3: 20.
17) Sato, S., S. Nagata, T. Imura, T. Fukuoka, T. Morita, Y. Takahashi, Y. Kondo, D. Kitamoto, and H. Habe. 2016. Synthesis and characterization of dioctanoyl glycerate as water-soluble trypsin inhibitor. J. Oleo Sci. 65: 251–256.
18) Zhou, C.-H., J.N. Beltramini, Y.-X. Fan, and G.Q. Lu. 2008. Chemoselective catalytic conversion of glycerol as a biorenewable source to valuable commodity chemicals. Chem. Soc. Rev. 37: 527–549.