Fatty Acid Species of Bio-Active Lysophosphatidic acid from Haploid Myxoamoebae of *Physarum Polycephalum*

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Abstract

Fatty acid species of bio-active lysophosphatidic acid (LPA) fraction isolated from haploid myxoamoebae of *Physarum polycephalum* were analyzed. *Physarum* LPA fraction was purified by a combination of thin layer, and column chromatographies. The alkaline hydrolysates of LPA were subjected to tandem mass spectrometry. The species of fatty acid of *Physarum* LPA were determined as hexadecanoic acid (palmitic acid), cyclopropane-containing hexadecanoic acid, 11-octadecanoic acid (vaccenoic acid), and cyclopropane-containing octadecanoic acid. The molar ratio of these four species was 100 : 75 : 35 : 40.

Introduction

The involvement of membrane phospholipids in the control of cell proliferation and DNA synthesis were discussed for the last decade [1–7]. Recently, we isolated a novel, specific DNA polymerase α inhibitor from phospholipid fraction of haploid myxoamoebae of a true slime mold, *Physarum polycephalum*. The structural studies suggested that this inhibitor was a novel lysophosphatidic acid (LPA) composed of cyclic phosphate and cyclopropane-containing hexadecanoic acid. We named this substance PHYLPA, and it showed unusual effects on cell proliferation and on DNA polymerase α activity [8].

LPA is a group of the simplest natural phospholipids, and is supposed to be a key intermediate in the biosynthesis of glycerolipids [9–11]. Recently, van Corven *et al.* reported that LPA has marked growth-promoting effects when added to quiscent fibroblast [6] as has also been known for phosphatidic acid (PA) [4, 5, 12]. They suggested that LPA initiates at least three separate signaling cascades. First, it activates a pertussis

toxin-insensitive G protein mediating phosphoinositide hydrolysis with subsequent Ca^{2+} mobilization and stimulation of protein kinase C. Second, it initiates the release of arachidonic acid in a GTP-dependent manner, but independent of prior phosphoinositide hydrolysis. Third, it activates a pertussis toxin-sensitive G protein mediating inhibition of adenylate cyclase.

Compared to these reports, our PHYLPA shows opposite effects in its mode of action both on cell proliferation and on DNA synthesis. These differences are considered to be the result of its unusual structure. Thus it is very valuable to study the molecular mechanism of the inhibitory action of PHYLPA.

Based on these interests, some other species of *Physarum* LPA were isolated and the fatty acid moiety of them were analyzed.

Experimental Procedures

Haploid myxoamoebae of a true slime mold *Physarum polycephalum* were cultured in the dark at 24°C on a lawn of *Aerobactor aerogenes*, on agar medium as described before [13, 14].

Physarum LPA fraction was purified essentially as described [8]. Myxoamoebae of *Physarum polycephalum* harvested after 5 day culture of 200 plates (18-cm diameter) were washed with distilled water and extracted with 10 vols. of methanol, chloroform/methanol (1 : 2), chloroform/methanol (1 : 1) and chloroform/methanol (2 : 1), successively. The combined extracts were evaporated and dissolved in chloroform/methanol (1 : 1), and subjected to DEAE-Sephadex column chromatography equilibrated with the same solvent. The unadsorbed fraction was pooled, concentrated and subjected to preparative TLC on silica gel 60 plates in the solvent systems I–IV : I, chloroform/methanol/water (60 : 40 : 9); II, chloroform/methanol/acetic acid/acetone/water (10 : 2 : 2 : 4 : 1); III, butanol/acetic acid/water (60 : 20 : 20); IV, diisobutyl ketone/acetic acid/water (40 : 25 : 4). Rf values of LPA fraction were 0.55–0.60 for the solvent I, 0.45–0.50 for the solvent II, 0.65–0.80 for the solvent III, and 0.50–0.60 for the solvent IV. LPA fraction was then applied to a column of Sephadex G-15 equilibrated with chloroform/methanol (1 : 1) and eluted with the same solvent. By this procedure, 1.5 mg of the LPA fraction was obtained.

The LPA fraction was subjected alkaline hydrolysis in 5N NaOH at 70°C for 5 h, and neutralized with the addition of HCl. Then the hydrolysates were extracted with diethylether, and subjected to fast atom bombardment mass spectrometry (FAB/MS). The structurally useful fragment ions were produced upon collisional activation (CA) from the parent ion provided by FAB ionization. The collisionally activated dissociation

(CAD) spectra of the fragments were taken by activating the parent ion in the second field free region using helium as the target gas and by scanning the electric field. 10 scans were averaged for each spectrum. Data were analyzed according to Cerny *et al.* [15].

Results and Discussion

The LPA fraction purified from haploid myxoamoebae of a true slime mold *Physarum polycephalum* was hydrolyzed and tandem mass spectrometry (MS/MS) in conjugation with FAB was performed. By the negative-ion FAB/MS of the hydrolysates, $[M-H]^-$ ions at *m/z* 255, 267, 281 and 295 were detected (Fig. 1), then the CAD spectrum of each $[M-H]^-$ were taken (Fig. 2). The CAD spectra of *m/z* 267 and 295 contained a pattern of abundant fragment ions, (a) and (b), resulting from β-cleavages to the three membered ring with four smaller peaks between them. Less intensity of the peak which is 14 u higher in mass than the peak (a) is also a characteristic feature of cyclopropane-containing fatty acid [1, 16]. The spectra of these four species of the fatty acid were analyzed according to Cerny *et al.* [15], then the fatty acid components of *Physarum* LPA were determined as hexadecanoic acid, cyclopropane-containing hexadecanoic acid,

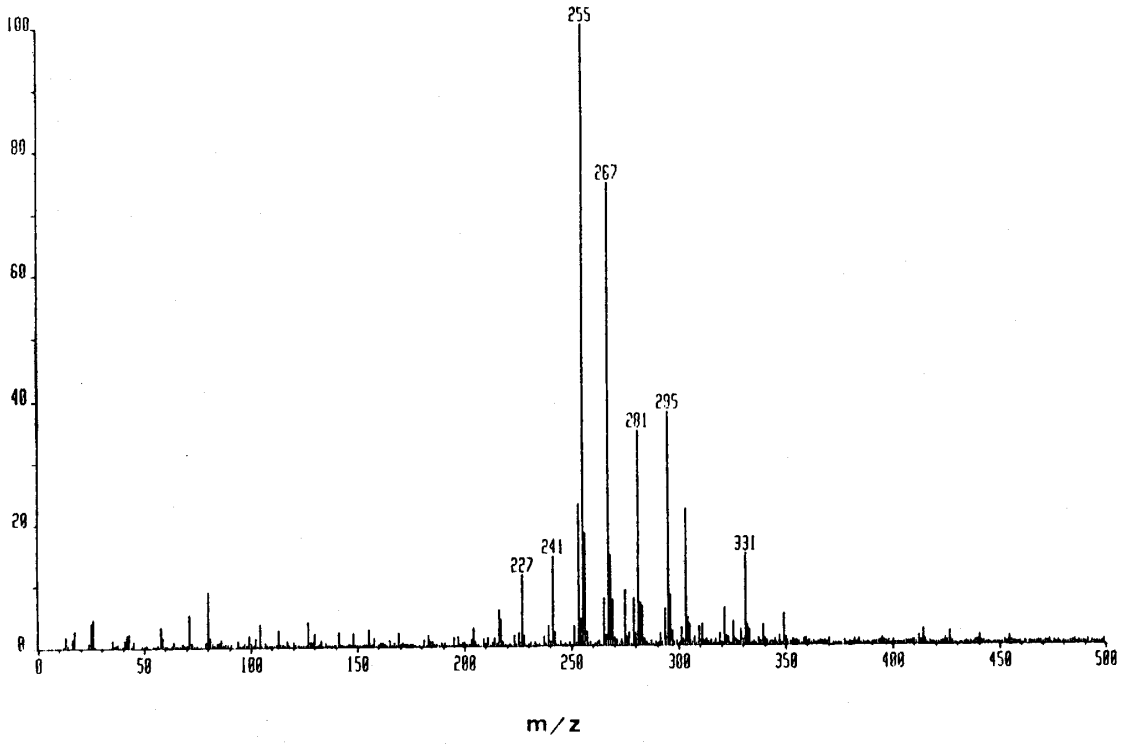

Fig. 1. Fast atom bombardment mass spectrometry (FAB/MS) of the hydrolysates of *Physarum* LPA. FAB/MS was performed on VG ZAB-HF with a matrix of diethanolamine, and negative ions were detected.

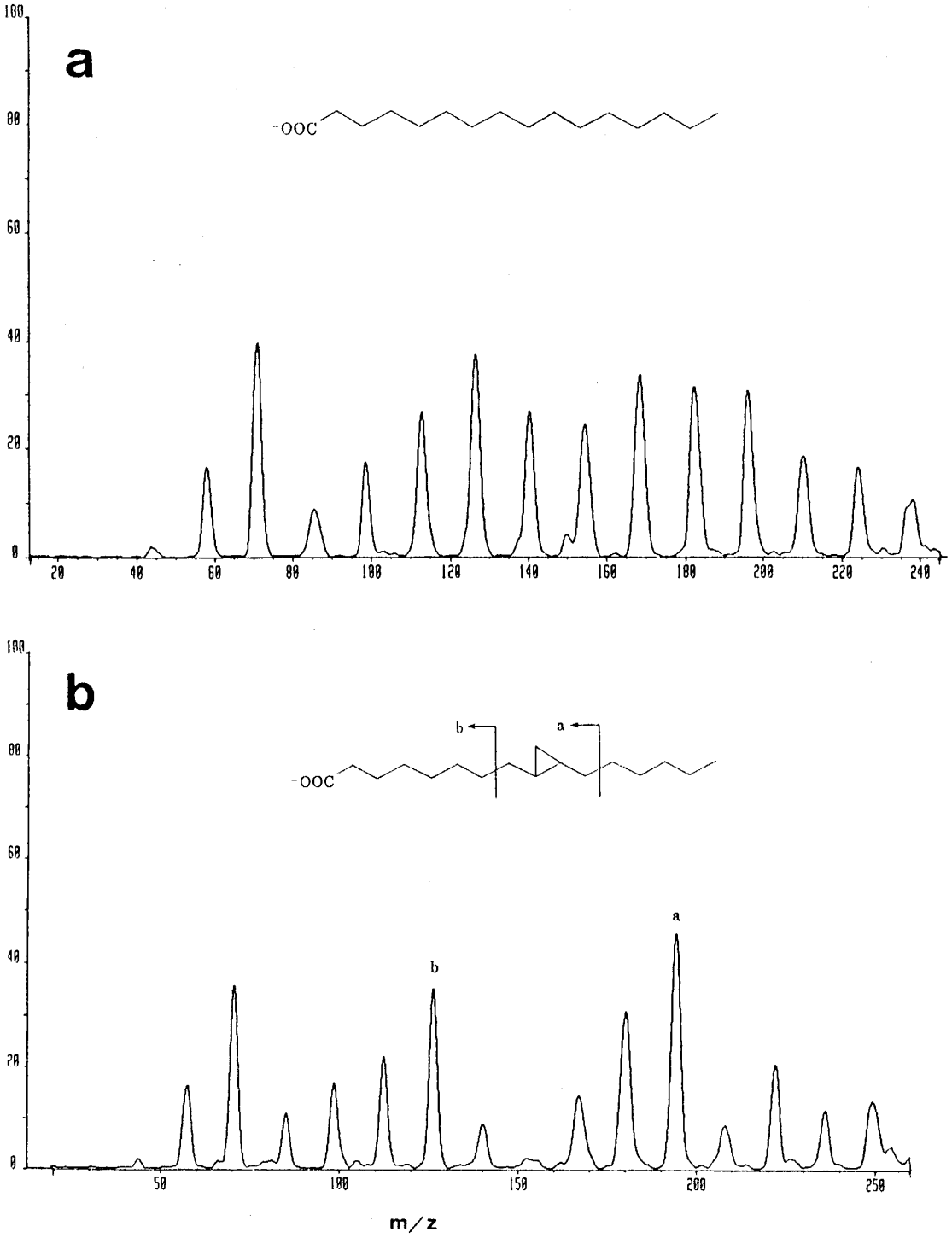

Fig. 2. a, b.

11-octadecanoic acid and cyclopropane-containing octadecanoic acid, respectively (Fig. 2a-d). From the result of FAB/MS, the molar ratio of these fatty acids were roughly estimated to be 100 : 75 : 35 : 40.

We are now interested in the relationship between the structure and the activity of these substances and the molecular mechanism of the bio-

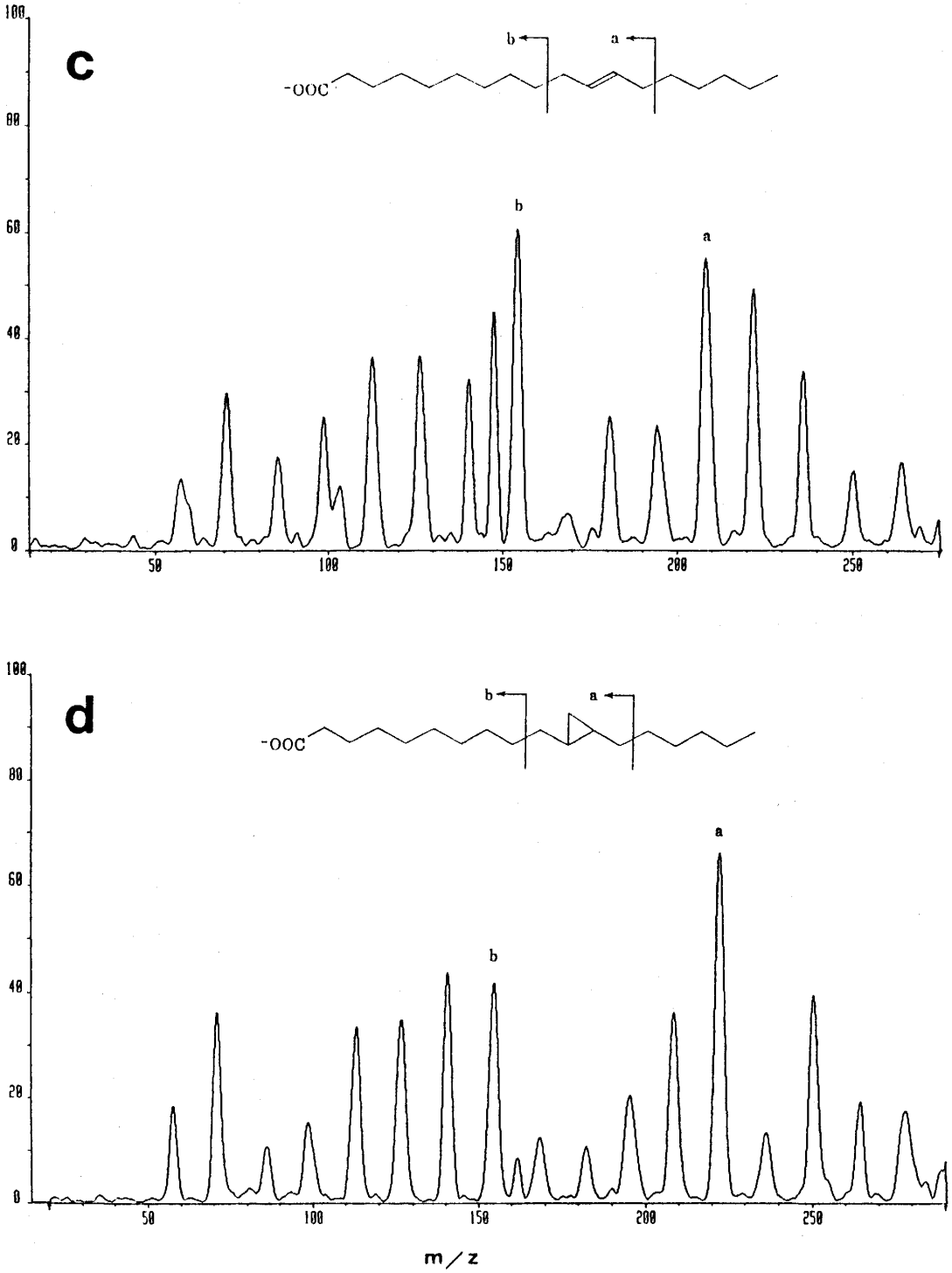

Fig. 2. c, d.

Fig. 2. Negative ion collisionally activated dissociation (CAD) spectra of the (a) *m/z* 255, (b) *m/z* 267, (c) *m/z* 281 and (d) *m/z* 295 carbohydrate fragment ions from the purified *Physarum* LPA.

logical effect of them. So, the efforts to resolve these problems are under way in our laboratory.

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References

[1] Otonaess, A.B., Krokan, A., Bzorklid, E. and Pryz, H. (1976): Biochim. Biophys. Acta **454**, 193-206.
[2] Coco, L., Maraldi, N.R. and Manzoli, F.A. (1980): Biochem. Biophys. Res. Commun. **96**, 890-898.
[3] Newport, J. (1987): Cell 48, 205-217.
[4] Moolenaar, W.H., Kruijer, W., Tilley, B.C., Verlaan, I., Bierman, A.J. and de Laat, S.W. (1986): Nature **323**, 171-173.
[5] Yu, C.-L., Tsai, M.-H. and Stacey, D.W. (1988): Cell **52**, 63-71.
[6] van Corven, E.J., Groenink, A., Jalink, K., Eicholtz, T. and Moolenaar, W.H. (1989): Cell **59**, 45-54.
[7] Knauss, T.C., Jaffer, F.E. and Abboud, H.E. (1990): J. Biol. Chem. **265**, 14457-14463.
[8] Murakami-Murofushi, K., Shioda, M., Kaji, K., Yoshida, S. and Murofushi, H. (1992): J. Biol. Chem. **267**, 21512-21517.
[9] Kennedy, E.P. (1962): Harvey Lec. **57**, 143-171.
[10] Bell, R.M. and Coleman, R.A. (1980): Annu. Rev. Biochem. **49**, 459-487.
[11] Hajra, A.K., Ghosh, M.K., Webber, K.O. and Datta, N.S. (1980): in *Enzymes of lipid metabolism II* (Freysz, L., Dreyfus, H., Massarelli, R., and Gatt, S., eds.), pp. 199-207, Plenum Press, New York.
[12] Siegmann, D. (1987): Biochem. Biophys. Res. Commun. **145**, 228-233.
[13] Taniguchi, M., Yamazaki, K. and Ohta, J. (1978): Cell Struct. Funct. **3**, 181-190.
[14] Murakami-Murofushi, K., Hiratsuka, A. and Ohta, J. (1984): Cell Struct. Funct. **9**, 311-315.
[15] Cerny, R.L., Tomer, K.B. and Gross, M.L. (1986): Organic Mass Spectrometry **21**, 655-660.
[16] Murakami-Murofushi, K., Takahashi, Y., Shimada, Y., Shioda, M. and Murofushi, H. (1993): in preparation.