Phagostimulant Activity of Phosphatidylcholine Molecular Species for Young Abalone *Haliotis discus hannai*

Yasuhiro Ando, Jun-ichi Nakamura, and Toru Ota

Department of Marine Bioresources Chemistry, Faculty of Fisheries, Hokkaido University, Minato, Hakodate 041, Japan

(Received December 13, 1996)

Key words: feeding stimulant, phagostimulant, phosphatidylcholine, fatty acid, abalone

Several glycerolipids such as phosphatidylcholines (PC), digalactosyldiacylglycerols (DGDG), 1,2-diacylglycerol-4'-*O*-(*N,N,N*-trimethyl)-homoserines (DGTH), 1-monoacylglycerol-4'-*O*-(*N,N,N*-trimethyl)-homoserines (MGTH), and 6-sulfoquinovosyldiacylglycerols (SQDG) have been isolated as potent feeding stimulants for the abalone *Haliotis discus* and the sea urchin *Strongylocentrotus intermedius* from the methanol extracts of some marine algae.¹⁻⁷⁾ These glycerolipids were mixtures of a number of molecular species. Saturated and unsaturated fatty acids (C₁₄–C₂₂) were major non-polar components located in the *sn*-1 and *sn*-2 positions of glycerol backbone. As far as the authors know, there have been few data on structure-activity relationships focused on the fatty acid moieties of the glycerolipids. In this study, phagostimulant activities for young abalone *Haliotis discus hannai* were compared among the commercially available molecular species of PC.

Thirteen synthetic molecular species of PC (purities, >99%) containing palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids were purchased from Avanti Polar-Lipids (Alabaster, AL, USA) and Sigma Chemical (St. Louis, MO, USA). DGDG originated from wheat were obtained from Serdary Research Labs (Englewood Cliffs, NJ, USA). All of the lipids were used without further purification. Young abalone *Haliotis discus hannai* (shell length, about 4 cm) were supplied by Hokkaido Institute of Mariculture (Hokkaido, Japan). The abalone were fed on fresh or air-dried brown alga *Laminaria japonica*.

Phagostimulant activity of the PC molecular species was evaluated by the bioassay procedure of Sakata *et al.*,⁵⁾ namely the Avicel plate method, after appropriate modifications as follows. Five to seven individuals of abalone were kept in each of three test aquariums (37 × 25 × 12 cm) containing 6 l of artificial sea water (3.5%). The sea water was continuously aerated, circulated, and filtrated through coral sands. Temperatures were maintained at 19–20°C in an incubator. Light and dark conditions were controlled at 12L/12D. The test abalone were starved for two days before the assay. Each of the test PC molecular species was dissolved in methanol at a concentration of 1.0 mg/ml, and then 40 µl of the solution was applied on a sample zone (2.3 cm in diameter) drawn on a Funacel SF crystalline cellulose thin-layer plate (Funakoshi, Tokyo, Japan). The test plates were left over-

night at the bottom of the test aquarium. The phagostimulant activity was expressed as the ratio of the area bitten off to that of the total area.

The assay was carried out for four combinations of the PC molecular species. Wheat DGDG were used as a positive control. DGDG were reported to show strong activity at high reproducibility.⁵⁾ Table 1 shows the phagostimulant activities of PC molecular species. The highest value was always observed for 1,2-dilinoleoyl-*sn*-glycerophosphatidylcholine (18:2–18:2) in each combination. Comparable values were obtained for 18:0–18:2, 18:1–18:2, 18:1–16:0, and 16:0–18:1; subsequent higher values for 16:0–18:2, 18:1–18:1, 18:1–18:0, and 18:0–18:1; and the lowest values for 16:0–16:0, 18:0–18:0, 18:0–16:0, and 16:0–18:0. In all the combinations, the phagostimulant activities were significantly different

Table 1. Phagostimulant activity of phosphatidylcholine molecular species for young abalone *Haliotis discus hannai**¹

	PC applied	% of area bitten off* ²
(A)* n=12	18:2–18:2	41 ± 9
	18:0–18:2	42 ± 9
	16:0–18:2	34 ± 9
	16:0–16:0	7 ± 2
	MeOH	2 ± 1
(B)** n=12	18:2–18:2	53 ± 10
	18:1–18:1	33 ± 10
	18:0–18:0	6 ± 1
	16:0–16:0	13 ± 3
	MeOH	6 ± 4
(C)** n=12	18:2–18:2	68 ± 8
	18:1–18:2	56 ± 10
	18:1–18:0	32 ± 8
	18:0–18:1	32 ± 10
	MeOH	7 ± 6
(D)** n=15	18:2–18:2	77 ± 10
	18:1–16:0	71 ± 11
	16:0–18:1	73 ± 11
	18:0–16:0	38 ± 10
	16:0–18:0	45 ± 10
	MeOH	29 ± 10

*¹ Activities of the positive control (wheat DGDG) were observed as follows: (A) 69 ± 9, (B) 71 ± 11, (C) 87 ± 6, and (D) 86 ± 8.

*² Mean ± standard error.

*** Significantly different among the PC molecular species ($p < 0.05^*$ and $p < 0.01^{**}$).

among the molecular species. Higher values were generally obtained for the unsaturated molecular species rather than saturated ones. There were also significant differences ($p < 0.001$ or $p < 0.01$) in the activities among the molecular species different in unsaturation of fatty acid moieties. Thus, the phagostimulant activity of PC was found to be related to the structure of the non-polar moieties, *i.e.*, the unsaturation in the fatty acids.

Sakata *et al.*⁷⁾ observed the activity of perhydrogenated PC similar to that of the original. The activities were assayed on separate test plates. In the present study, different activities were revealed by the application of saturated and unsaturated molecular species to one test plate.

Abalone in nature feed on marine algae. Lipids of algae generally contain high levels of polyunsaturated fatty acids including 18:2, 18:3, 18:4, 20:4, and 20:5.⁸⁻¹⁰⁾ Such unsaturated fatty acids are esterified to phagostimulant glycerolipids as major fatty acid components.¹⁰⁻¹²⁾ Fatty acids in such glycerolipids seem to play important roles in the feeding behavior of the abalone.

The authors thank Messrs. Yoshiaki Niihara, Takashi Sugimoto, and Hiroshi Hoshikawa, Hokkaido Institute of Mariculture, for providing the abalones and useful advice during this work.

References

- 1) K. Sakata, T. Itoh, and K. Ina: *Agric. Biol. Chem.*, **48**, 425-429 (1984).
- 2) K. Sakata, M. Tsuge, and K. Ina: *Mar. Biol.*, **91**, 509-511 (1986).
- 3) K. Sakata, T. Kusaka, T. Kamiya, and K. Ina: *Nippon Suisan Gakkaishi*, **54**, 1715-1718 (1988).
- 4) K. Sakata, T. Sakura, and K. Ina: *J. Chem. Ecol.*, **14**, 1405-1416 (1988).
- 5) K. Sakata, K. Kato, Y. Iwase, H. Okada, K. Ina, and Y. Machiguchi: *J. Chem. Ecol.*, **17**, 185-193 (1991).
- 6) K. Sakata and K. Ina: *Agric. Biol. Chem.*, **47**, 2957-2960 (1983).
- 7) K. Sakata and K. Ina: *Nippon Suisan Gakkaishi*, **51**, 659-665 (1985).
- 8) R. G. Ackman: in "New Sources of Fats and Oils" (ed. by E. H. Pryde, L. H. Princen, and K. D. Mukherjee), American Oil Chemists' Society, Champaign, IL, 1981, pp. 189-220.
- 9) T. Takagi, M. Asahi, and Y. Itabashi: *Yukagaku*, **34**, 1008-1012 (1985).
- 10) M. Kayama, S. Araki, and S. Sato: in "Marine Biogenic Lipids, Fats, and Oils" Vol. 2 (ed. by R. G. Ackman), CRC Press, Boca Raton, Florida, 1989, pp. 3-48.
- 11) J. Rulíkötter, E. Heinz, and A. P. Tulloch: *Z. Pflanzenphysiol. Bd.*, **76**, 163-175 (1975).
- 12) S. Araki, T. Sakurai, A. Kawaguchi, and N. Murata: *Plant. Cell Physiol.*, **28**, 761-766 (1987).