Glycerolipid Acyl Hydrolase Activity in the Brown Alga *Cladosiphon okamuranus* TOKIDA

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Received December 18, 2002; Accepted May 21, 2003

The brown alga, *Cladosiphon okamuranus* TOKIDA (Japanese name, Okinawamozuku), is frequently consumed as an edible seaweed in Japan, and is known to contain a large amount of fucoidan which is a polysaccharide having such physiological functions as a serum cholesterol-normalizing effect and anti-ulcer activity.1–3) In addition to fucoidan, other constituents such as lipids would also play an important role in the alga as a functional food, although they seem not to have been sufficiently studied.

There are only a few reports on the lipid components of *C. okamuranus*. Saito et al. have observed that natural and cultured *C. okamuranus* contained a large amount of free fatty acid (FFA) which constituted about 50\% of the total lipids (unpublished results). Kakisawa et al. have isolated a polyunsaturated FFA (18:4n-3) from the methanol extract of *C. okamuranus* as an allelopathic substance which killed other seaweeds and microalgae or inhibited their growth.4) These results demonstrate that the lipids of *C. okamuranus* can be characterized by the presence of a significant amount of FFA in the lipids. However, there have not yet been any reports on the formation of FFA in the alga. In the present study, we found glycerolipid acyl hydrolase activity in a crude enzyme preparation from *C. okamuranus* which could release FFA from such glycolipids and phospholipids as monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and phosphatidylcholine (PC).

The brown alga, *C. okamuranus*, which was originally grown in Okinawa and packed in salt water without any additives, was purchased at a market in Hakodate. The lipids were extracted by the Bligh and Dyer method,5) and triacylglycerol (TAG), FFA, free sterol (ST), and polar lipids (PL), including glycolipids and phospholipids, were quantified by thin-layer chromatography with flame-ionization detection (TLC-FID) on Chromarod S-III, using an Iatroscan TH-10 instrument (Iatron Labs, Tokyo, Japan).6) A qualitative analysis of the lipid classes was carried out by analytical TLC on silica gel 60F\textsubscript{254} aluminium sheets by using chloroform–methanol–water–ethyl acetate–2-propanol (5:2:1:5:5, by vol.) as the developing solvent. The fatty acid composition was determined by open-tubular gas chromatography on an Omegawax 320 column (30 m × 0.32 mm i.d.).7) MGDG, DGDG, sulfoquinovosyldiacylglycerol (SQDG), and phosphatidylglycerol (PG), which had been isolated from parsley and spinach leaf lipids by preparative TLC,8) were used as the substrates for the enzyme reaction, together with synthetic PC (1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine; Sigma, MO, U.S.A.). These substrates gave single spots by silicic acid TLC under the conditions already described, showing no presence of FFA. The acyl hydrolase activities were assayed by measuring the quantity of FFA released from each substrate in the presence of a crude enzyme preparation, as described previously.9) In brief, acetone powder (10 mg) prepared from *C. okamuranus* according to the procedure described by Bier\textsuperscript{9} was added to a 0.05 M sodium phosphate buffer solution (pH 7.0,
Fig. 1. TLC Chromatogram of the Total Lipids from Cladosiphon okamuranus.

Spots were detected by heating at 100°C after spraying with an orcinol-sulfonic acid reagent. The other TLC conditions and abbreviations as given in the text.

1 ml) containing 1% Triton X-100, the mixture being stirred at 20°C for 1 h (500 rpm). The suspension was centrifuged at 1500×g for 5 min, and the resulting supernatant was used for the assay of acyl hydrolase activity. The released FFA was determined by high-performance liquid chromatography with fluorescence detection after conversion to the 9-anthrylmethyl ester. The value was corrected by subtracting the small quantity of FFA released from the crude enzyme preparation.

The lipid content of C. okamuranus was 1.3% of the dry weight, in which FFA and PL (mainly glycolipids) constituted 45.1% and 39.9%, respectively. Small amounts of sterol ester (SE, 1.2%), TAG (2.4%), 1,3-diaclylglycerol (1,3-DAG, 1.8%), 1,2-DAG (3.1%), and ST (6.5%) were also found by TLC-FID (chromatograms not shown). PL consisted of several components (Fig. 1), MGDG (Rf, 0.61), DGDG (Rf, 0.32), and SQDG and PC (Rf, 0.20) being the major ones, although they were not quantitated because of their poor separation by TLC-FID. PC (Rf, 0.03) was a minor component. The presence of appreciable amounts of lyso-compounds, that is, monogalactosylmonoaurylglycerol (MGMG, Rf, 0.41) and digalactosylmonoaurylglycerol (DGMG, Rf, 0.15), suggests that FFA in C. okamuranus was mainly produced by galactolipase which hydrolyzed the ester bonds of the MGDG and DGDG molecules. The FFA composition of C. okamuranus was similar to that of PL. The predominant component of FFA and PL was 16:0 (30.1% in FFA; 22.9% in PL), and the lesser components were 14:0 (5.2%; 7.2%), 18:1n-9 (7.8%; 9.6%), 18:2n-6 (7.8%; 8.9%), 18:3n-3 (13.2%; 5.3%), 18:4n-3 (11.1%; 14.2%), 20:4n-6 (7.5%; 8.9%), and 20:5n-3 (8.6%; 6.3%). These results imply that the enzyme in C. okamuranus may have hydrolyzed both the saturated and unsaturated acyl groups of the MGDG and DGDG molecules.

The crude enzyme preparation from C. okamuranus released significant amounts of 16:3n-3 and 18:3n-3 from parsley MGDG, of which the major molecular species (sn-1/sn-2) was 18:3n-3/16:3n-3 (88% of total MGDG). This suggests that FFA in C. okamuranus would have been formed by the cleavage of both ester bonds of MGDG by the action of such acyl hydrolases as galactolipase. The optimum temperature for the crude enzyme preparation was 37°C at pH 7.0, proving the occurrence of enzymatic reactions in the buffered solution (Fig. 2A). The optimum pH appeared to be near 9.0, although accurate measurements would be required to confirm this (Fig. 2B). These results clearly show the action of glycerolipid acyl hydrolase activity in the alga. High acyl hydrolase activity at pH 9.0 has also been reported in the red-tide alga, Chattonella marina, although glyco- and phospholipid acyl hydrolases in various plant tissues have pH optima in the acidic or neutral range.

Figure 3 shows the acyl hydrolase activity of the crude enzyme preparation from C. okamuranus toward different lipid classes. The enzyme preparation had the highest activity toward MGDG, whereas it had lower activity toward DGDG. Although high phospholipase A activity was also observed, the PC content was much less than that of glycolipids in the alga (see Fig. 1). These results indicate that FFA in C. okamuranus was mainly released from MGDG and DGDG by acyl hydrolases such as galactolipase that was present in the alga. The presence of significant amounts of the lyso-compounds, MGG and DGMG, also suggests the presence of this enzyme in C. okamuranus. Higher activity toward MGDG and PC than toward other lipid classes has also been reported for the case of C. marina.

The lower activity toward spinach leaf PG than toward synthetic PC (Fig. 2) would have been due to the large amount of trans-16:1n-13 in PG (30% of
the total fatty acids) which would have been resistant to hydrolysis. Sahsah et al. have also observed that the galactolipase activity in cowpea leaves was lower toward PG, with 16:1 comprising 23% of the total fatty acids, than toward PC. Both proteins of galactolipase and phospholipase may have been present in the crude enzyme preparation of C. okamuranus. Purification and characterization of galactolipase in C. okamuranus are in progress.

To our knowledge, this is the first report on the presence of acyl-hydrolase activity in seaweeds. The results presented here should be helpful in promoting work on the production and physiological significance of FFA in C. okamuranus.

Acknowledgment

The authors thank Dr. Hiroaki Saito (National Research Institute of Fisheries Science) for valuable discussions on FFA of C. okamuranus.

References


Fig. 2. Acyl Hydrolase Activities of the Crude Enzyme Preparation from Cladosiphon okamuranus.
(A) Effect of temperature on the activity at pH 7.0. (B) Effect of pH on the activity at 37°C. Data (mean ± SD, n = 3) are expressed as the sum of 16:3n-3 and 18:3n-3 released from parsley MGDG.

Fig. 3. Acyl Hydrolase Activities toward Different Lipid Classes of the Crude Enzyme Preparation from Cladosiphon okamuranus.
The enzyme reaction was carried out in a 0.05 M Tris-HCl buffer (37°C, pH 9.0). See the text for abbreviations.

