Biochimica et Biophysica Acta 1801 (2010) 508–516

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Lipolysis of natural long chain and synthetic medium chain galactolipids by pancreatic lipase-related protein 2☆

Sawsan Amara [a], Nathalie Barouh [b], Jérôme Lecomte [b], Dominique Lafont [c], Sylvie Robert [a], Pierre Villeneuve [b], Alain De Caro [a], Frédéric Carrière [a,*]

[a] CNRS—Aix-Marseille Université—Enzymologie Interfaciale et Physiologie de la Lipolyse—UPR 9025, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France
[b] CIRAD, UMR IATE, F-34398 Montpellier, France
[c] Laboratoire de Chimie Organique II-Glycochimie, ICBMS UMR 5246, CNRS—Université Claude Bernard Lyon 1, Université de Lyon, Bâtiment Curien, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France

ARTICLE INFO

Article history:
Received 12 November 2009
Received in revised form 17 December 2009
Accepted 3 January 2010
Available online 18 January 2010

Keywords:
Bile salt
Digalactosyl diacylglycerol
Fatty acid
Galactolipase
Galactolipids
Gas chromatography
Mixed micelle
Monogalactosyl diacylglycerol
Pancreatic lipase-related protein 2
Thin layer chromatography

ABSTRACT

Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are the most abundant lipids in nature, mainly as important components of plant leaves and chloroplast membranes. Pancreatic lipase-related protein 2 (PLRP2) was previously found to express galactolipase activity, and it is assumed to be the main enzyme involved in the digestion of these common vegetable lipids in the gastrointestinal tract. Most of the previous *in vitro* studies were however performed with medium chain synthetic galactolipids as substrates. It was shown here that recombinant guinea pig (*Cavia porcellus*) as well as human PLRP2 hydrolyzed at high rates natural DGDG and MGDG extracted from spinach leaves. Their specific activities were estimated by combining the pH-stat technique, thin layer chromatography coupled to scanning densitometry and gas chromatography. The optimum assay conditions for hydrolysis of these natural long chain galactolipids were investigated and the optimum bile salt to substrate ratio was found to be different from that established with synthetic medium chains MGDG and DGDG. Nevertheless the length of acyl chains and the nature of the galactosyl polar head of the galactolipid did not have major effects on the specific activities of PLRP2, which were found to be very high on both medium chain [1786 ± 100 to 5420 ± 85 U/mg] and long chain [1756 ± 208 to 4167 ± 167 U/mg] galactolipids. Fatty acid composition analysis of natural MGDG, DGDG and their lipolysis products revealed that PLRP2 only hydrolyzed one ester bond at the *sn-1* position of galactolipids. PLRP2 might be used to produce lipid and free fatty acid fractions enriched in either 16:3 $n-3$ or 18:3 $n-3$ fatty acids, both found at high levels in galactolipids.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

In higher plants, the photosynthesis process takes place in specific organelles, the chloroplasts and especially at the level of their thylakoid membranes. Proteins and lipids account for 60–65% and 35–40% (w/w) of these membranes, depending on growth conditions. The lipid composition of the thylakoids is unique among eukaryotic cellular membranes and about 70–80% of their total lipids are neutral galactosyldiacylglycerols [1] (Fig. 1). Overall, plants like spinach contain a portion of galactolipids (59.5%) much larger than that of phospholipids (22.4%) among their fat-soluble components [2]. Galactolipids are also much more abundant in the plant kingdom than triacylglycerols, the main lipid molecules (99%) found in vegetable oils produced from oil seeds (soybean, rapeseed, sunflower, maize, and peanut) and plant fruits (palm and olive).

The monogalactosyldiacylglycerol (MGDG) is generally found at higher levels than digalactosyldiacylglycerol (DGDG) in photosynthetic tissues (about 51% MGDG versus 26% DGDG). Galactolipids are also present in nonphotosynthetic tissues such as potato tuber, fruits, and seeds [3]. Other galactolipids in lower amounts have also been found in plants such as tri-galactosyldiacylglycerol, tetra-galactosyldiacylglycerols [4,5] and sulfoquinosyldiacylglycerol (SQDG), a negatively charged (acid) sulfolipid present in the thylakoid membrane. In spinach, the MGDG/DGDG/SQDG ratio was found to be 57:32:11 (w/w/w) [6]. Galactolipids share some structural characteristics with phospholipids, and the physico-chemical properties of DGDG are close to those of phosphatidylcholine, with the ability to form spontaneously bilayers in water (liposomes and Lα lamellar structures).

The galactose polar head is bound to the *sn-3* position of the glycerol backbone, with the first galactose residue in β-anomeric linkage. In DGDG, the head group is characterized by a terminal α-galactose linked to the inner β-galactose residue. In contrast to the

☆ This article is dedicated to the memory of our colleague and friend Dr. Alain De Caro who died on July 20th, 2009.

* Corresponding author. Tel.: +33 491 164 134; fax: +33 491 715 857.
E-mail address: carriere@ifr88.cnrs-mrs.fr (F. Carrière).

1388-1981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2010.01.003

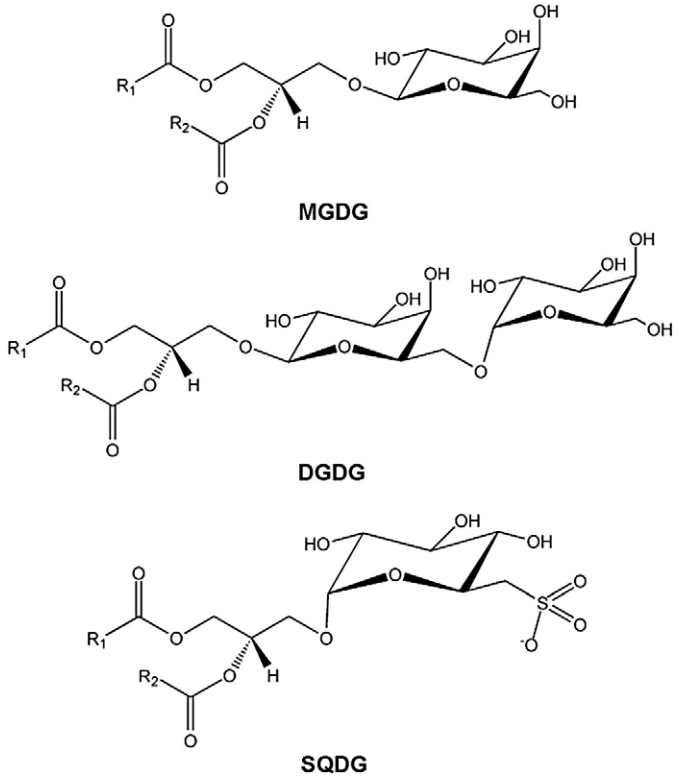

Fig. 1. Chemical structures of 1,2-diacyl-3-O-β-D-galactosyl-sn-glycerol (MGDG), 1,2-diacyl-3-O-(6-O-α-D-galactosyl-β-D-galactosyl)-sn-glycerol (DGDG), and 1,2-diacyl-3-(6-sulfo-α-D-quinovosyl)-sn-glycerol (SQDG).

conserved head group structures of MGDG and DGDG, the fatty acids of galactolipids exhibit a high variability in chain lengths, degree of unsaturation and distribution at the *sn-1* and *sn-2* position of the glycerol backbone [7].

An interesting feature of galactolipids is their natural enrichment in long chain polyunsaturated fatty acids such as linolenic acid (18:3 $n-3$), or hexadecatrienoic acid (16:3 $n-3$) which is found in high amounts in MGDG [8].

Although it was thought for a long time that galactolipids were not hydrolyzed by mammalian digestive enzymes, it has now been demonstrated that galactolipases are present in pancreatic secretion [9,10]. As early as 1974, it was observed that a saline extract of acetone-dried powder of sheep pancreas was able to catalyse acyl ester hydrolysis of spinach leaf MGDG [11]. Attempts to purify a monogalactosyl diglyceride acyl hydrolase gave a partially purified enzyme, which activity was stimulated by sodium deoxycholate. This partially purified protein also showed acyl ester hydrolysis activity towards methyl oleate, phosphatidylcholine and triacylglycerol. Sheep, rat and guinea pig tissues (pancreas and intestine) were compared and guinea pig tissues showed the highest activities towards both MGDG and DGDG. In all these species, the pancreas was found to contain a higher activity than the intestine.

It was shown some years later, using radioactive galactolipids, that the pancreatic lipase-related protein 2 from guinea pig (GPLRP2) displayed a high galactolipase activity whereas the classical human pancreatic lipase had no activity on galactolipids [12]. Using monomolecular films of synthetic MGDG and DGDG as substrates, it was also shown that rat and human PLRP2 (HPLRP2) were galactolipases [13–15].

The occurrence of PLRP2 in the pancreas of different species (mammalian and bird) was then investigated [16]. The pancreas of both omnivorous (human, mice and rat) and monogastric herbivorous animals (guinea pig, coypu, rabbit and horse) were found to contain PLRP2, while carnivorous (dogs and cats), ruminant herbivorous animals (ox, goat and sheep), pig and birds (ostrich and turkey) did not have any detectable PLRP2. The presence of PLRP2 was strictly associated with a galactolipase activity. The ruminants, monogastric herbivores and omnivores' diet daily contains plants and vegetables in various ratio and galactolipids are therefore ingested by all these species. As an example, guinea pigs might consume 700 mg of galactolipids a day on average, while humans only 200 mg a day [16]. Since galactolipids are normally absent in carnivorous animals' diet, it is likely that a relationship exists between the occurrence of PLRP2 and the diet. PLRP2 was not found in ruminants, but microbial enzymes might replace PLRP2 in these species.

A specific and continuous galactolipase assay using synthetic medium chain MGDG as substrate and the pH-stat technique for monitoring the release of fatty acids was recently described [17]. Synthetic MGDG had to be mixed with bile salts in a micellar form to provide optimum conditions for its hydrolysis by galactolipases. This assay was used to rank various digestive enzymes showing galactolipase activity, including PLRP2 and carboxyl ester hydrolases from both pancreas and mother milk. This study and previous ones [9,10,14] strongly suggested that HPLRP2 was the main enzyme involved in the digestion of galactolipids in humans. The activity of purified HPLRP2 on natural long chain galactolipids was however never measured and there was only one report on GPLRP2 activity on radiolabeled long chains MGDG and DGDG [12].

Based on previous knowledge obtained with medium chain MGDG [17], the aim of the present study was to measure the rate of hydrolysis by PLRP2 of natural long chain galactolipids extracted from spinach leaves and to compare this activity with those obtained with synthetic medium chains MGDG and DGDG, the latter compound being specifically synthesized for this study. The regioselectivity of PLRP2 was also deduced from fatty acid analysis.

2. Materials and methods

2.1. Chemicals

Anhydrous magnesium sulfate, anhydrous sodium sulfate, potassium chloride, sodium chloride, taurodeoxycholic acid sodium salt (NaTDC), cupric acetate monohydrate, orthophosphoric acid, iodine, boron trifluoride–methanol (MeOH–BF_3) solution (14% in methanol), sodium methoxide (MeONa) solution (ACS reagent, 0.5 M in methanol) and hydrochloric acid were purchased from Sigma Aldrich.

Thin layer silica gel 60 plates (10×20 cm from Merck) were used for the separation of the lipids. Silica gel 60 powder (70–230 mesh) (Sigma Aldrich, Steinheim, Germany) was used for galactolipids purification by column chromatography. All solvents were purchased from SDS (Peypin, France) and were of HPLC grade. Lipid standards (α-L-linolenic acid, digalactosyl diglyceride (DGDG) and galactosyl diglyceride (MGDG) isolated from wheat) were purchased from Sigma Aldrich.

2.2. Enzymes

Recombinant HPLRP2 (rHPLRP2) was produced in the yeast *Pichia pastoris* and purified as described previously [14,15]. Recombinant GPLRP2 (rGPLRP2) was expressed and purified from *Aspergillus orizae* [18]. The homogeneity of the various enzymes was routinely assessed by performing SDS-PAGE on 12% gels using Laemmli's procedure [19], N-terminal sequencing and MALDI-TOF mass spectrometry. The concentration of pure rHPLRP2 and rGPLRP2 was determined from the absorbance at 280 nm using $E^{1\%}_{1cm}=14$ and 13, respectively. Alternatively, protein concentrations were determined with good accuracy using Bradford's procedure [20], with Bio-Rad dye reagent and BSA as the protein standard.

Download English Version:

https://daneshyari.com/en/article/1949768

Download Persian Version:

https://daneshyari.com/article/1949768

Daneshyari.com