Food Chemistry 160 (2014) 61–66

Contents lists available at ScienceDirect

Food Chemistry

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

Pancreatic lipase selectively hydrolyses DPA over EPA and DHA due to location of double bonds in the fatty acid rather than regioselectivity

Taiwo O. Akanbi [a], Andrew J. Sinclair [b], Colin J. Barrow [a,*]

[a] Centre for Chemistry and Biotechnology, Deakin University, Locked Bag 20000, Geelong, VIC, Australia
[b] School of Medicine, Deakin University, Locked Bag 20000, Geelong, VIC, Australia

ARTICLE INFO

Article history:
Received 16 September 2013
Received in revised form 3 March 2014
Accepted 18 March 2014
Available online 26 March 2014

Keywords:
Pancreatic lipase
Docosapentaenoic acid
Seal oil
Regioselectivity
Polyunsaturated fatty acids

ABSTRACT

The enzymatic hydrolysis of canola, anchovy and seal oils with different types and amounts of polyunsaturated fatty acids was measured using porcine pancreatic lipase (PPL) to establish the fatty acid selectivity of PPL. Substrates were subjected to the same conditions of hydrolysis, with percent hydrolysis monitored using Iatroscan and fatty acid selectivity monitored using gas chromatography (GC). Regardless of their distribution on the glycerol backbone, as monitored by ^{13}C nuclear magnetic resonance (NMR), α-linolenic acid (ALA) and docosapentaenoic acid (DPA) were rapidly cleaved by PPL while eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and stearidonic acid (STA) were hydrolysed more slowly. Results show that PPL preferentially hydrolyses ALA and DPA over EPA, DHA and STA, and this selectivity is due to fatty acid rather than regioselectivity. The primary structural factor associated with resistance to PPL appears to be the distance of the first double bond from the ester linkage being hydrolysed.

1. Introduction

Pancreatic lipase, also known as triacylglycerol acylhydrolase (EC 3.1.1.3), is an important enzyme for the digestion of dietary fats (Arroyo, Sánchez-Muniz, Cuesta, Burguillo, & Sánchez-Montero, 1996). It cleaves dietary triacylglycerol (TAG) derivatives into monoacylglycerols (MAGs) and free fatty acids (FFAs) (Arroyo et al., 1996; Chapus, Rovery, Sarda, & Verger, 1988). The amino acid sequence of pancreatic lipase has been reported to be very similar among animals such as human, canine, porcine and rat (Kirchgessner, Svenson, Lusis, & Schotz, 1987; Wishart et al., 1993).

This enzyme has been used for studying the positional distribution of fatty acids on the glycerol backbone of oils, by hydrolysing some fatty acids in positions 1 and 3 (*sn*-1,3) and leaving those in position 2 (*sn*-2) unhydrolysed (Luddy, Barford, Herb, Magidman, & Riemenschneider, 1964). However, it was earlier found that pancreatic lipase can also hydrolyse fatty acids in the *sn*-2 position (Mattson & Volpenhein, 1961). Therefore, the positional selectivity of pancreatic lipase remains unclear as does the fatty acid selectivity, particularly with regard to omega-3 fatty acids.

Long-chain omega-3 fatty acids such as *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) have been reported to resist hydrolysis by pancreatic lipase owing to the closeness of a double bond to the carboxyl end (Bottino, Vandenburg, & Reiser, 1967). Bottino et al. (1967) identified their chemical structures, orientation of terminal methyl ends and the double bond positions as the major contributors to their resistance to hydrolysis by pancreatic lipase. However, investigation into the relative selectivity of pancreatic lipase to a broad range of polyunsaturated fatty acids from diverse sources is lacking. While fish oil is rich in EPA and DHA, oils of marine mammals such as seal are rich in EPA, DHA and DPA. Also, DPA makes a major contribution to omega-3 intake in some populations with relatively high intake of red meat, such as Australians, but there is little information on DPA sensitivity to hydrolysis by lipases, including PPL. There is no information on the relative selectivity of pancreatic lipase to DPA, as compared with EPA and DHA. Determining the relative selectivity toward these fatty acids is complicated by the differing positional distribution of each on the acyl glycerol backbone for fish and seal oils (Wanasundara & Shahidi, 1997). However, we have recently been able to determine fatty acid selectivity versus regioselectivity for a lipase by using a combination of Iatroscan, GC and ^{13}C NMR spectroscopy and here apply these methods to porcine pancreatic lipase action on oils rich in various omega 3 fatty acids (Akanbi, Adcock, & Barrow, 2013; Wanasundara & Shahidi, 1997).

Since positional selective lipases have been reported to be applicable in the production of omega-3 fatty acids such as DHA (Senanayake & Shahidi, 1999, 2002), it is possible that the selectivity

* Corresponding author. Tel.: +61 352271318; fax: +61 352271040.
E-mail address: colin.barrow@deakin.edu.au (C.J. Barrow).

http://dx.doi.org/10.1016/j.foodchem.2014.03.092
0308-8146/

of pancreatic lipase may be useful in studies aimed at concentrating omega-3 fatty acids. Several studies have shown that pancreatic lipases can be successfully immobilised for repeated reuse, an important criterion for cost effective application of these enzymes (Bagi, Simon, & Szajani, 1997; Bautista et al., 1999; Kılınç, Teke, Önal, & Telefoncu, 2006). In any event, determining omega-3 selectivity of pancreatic lipases will provide useful information on the function of these biologically important lipases.

Therefore, in this present study, we determined the selectivity of porcine pancreatic lipase (PPL) for polyunsaturated fatty acids, including the omega-3 fatty acids EPA, DHA, DPA, STA and ALA, using canola, anchovy and seal oils. We discuss the observed selectivity with regard to the impact of the chemical structures, chain lengths and the double bond positions of these polyunsaturated fatty acids. We use a combination of Iatroscan, GC and ^{13}C NMR to determine regioselectivity and fatty acid selectivity of PPL.

2. Materials and methods

2.1. Materials

Anchovy oil was provided by Ocean Nutrition Canada (now DSM) and seal oil was a gift from Professor Fereidoon Shahidi, Memorial University of Newfoundland, and canola oil was purchased from a local supermarket in Australia. Porcine pancreatic lipase was purchased from Sigma–Aldrich (Castle Hill, Australia). Capillary chromatography standards were purchased from Nu-Chek Prep (Elysian, MN, USA). All other chemicals were of analytical grade.

2.2. ^{13}C NMR spectroscopy

Quantitative ^{13}C NMR spectra of the unhydrolysed oils were recorded under continuous ^{1}H decoupling at 24 °C using a Brucker Avance 500 MHz. The spectra were collected on 0.5 g of the oil samples dissolved in 700 μL $CDCl_3$ (99.8% pure). In order to quantify the residue of each fatty acid at different positions, peak area ratios were analysed by integration and presented in percentages (Akanbi et al., 2013).

2.3. Lipase-catalysed hydrolysis of oil

Enzymatic hydrolysis was carried out following the method of Luddy et al. (1964) with some modifications. To a 5 mL flask containing 300 mg of oil were added 100 μL of 22% calcium chloride ($CaCl_2$) solution, 200 μL of 1 M tris(hydroxymethyl)aminomethane at pH 7.7 and 320 μL of 0.1% bile salts solution. The mixture was gently flushed with nitrogen and warmed in a water bath at 37 °C for 5 min before the addition of 10,000 units of PPL. Hydrolysis was thereafter carried out in the dark at 37 °C under nitrogen with magnetic stirring at 200 rpm and aliquots of sample were withdrawn at different time intervals until 50(±5)% hydrolysis degree was attained. The glycerol and free fatty (FFA) portions of each sample were obtained and separated by firstly extracting acylglycerols from the mixture with 10 mL of *n*-hexane after adding 3.5 mL of 0.5 M KOH (30% ethanol solution) to neutralise the fatty acid released during hydrolysis. FFA in the water layer were then extracted with 10 mL *n*-hexane after returning to acidic pH 1.0 with 4 M HCl, as described previously (Gamez-Meza et al., 2003).

2.4. Hydrolysis degree and lipid class analysis

Capillary chromatography with flame ionisation detector (Iatroscan MK5, Iatron Laboratories Inc., Tokyo, Japan) was used to determine the degree of hydrolysis. Portions of both the unhydrolysed and hydrolysed oil were analysed by Iatroscan as previously reported (Akanbi et al., 2013). Percent hydrolysis was determined using SIC-480 II software for multiple chromatogram processing, by comparing the percentage peak areas of the unhydrolysed and hydrolysed triacylglycerol (TAG). Capillary chromatography standards purchased from Nu-Chek Prep were used to identify each lipid class.

Table 1
Molar percentages of fatty acids at the *sn*-1,3 and *sn*-2 glycerol positions of anchovy and seal oils.

Position	Fatty acid assignment	Molar percentage (%)	
		Anchovy oil[a]	Seal oil[a]
sn-1,3	Saturated	23.3	19.7
	Monounsaturated, Δ9	16.0	25.3
	STA (C18:4), Δ6	6.5	3.0
	EPA (C20:5), Δ5	14.5	6.8
	DPA (C22:5), Δ7	2.1	4.2
	DHA (C22:6), Δ4	4.3	8.0
sn-2	Saturated	13.0	10.1
	Monounsaturated, Δ9	5.5	21.2
	STA (C18:4), Δ6	2.9	0.6
	EPA (C20:5), Δ5	3.5	0.6
	DPA (C22:5), Δ7	1.9	ND[b]
	DHA (C22:6), Δ4	6.6	0.5

[a] Values are means of three spectral fittings. For each spectrum, 6500 scans were collected.
[b] Not detected.

2.5. Analysis of fatty acid composition by gas chromatography

Fatty acids in both the unhydrolysed and hydrolysed portions of the oils were converted to methyl esters and the resulting fatty acid methyl esters (FAMEs) were analysed by an Agilent 6890

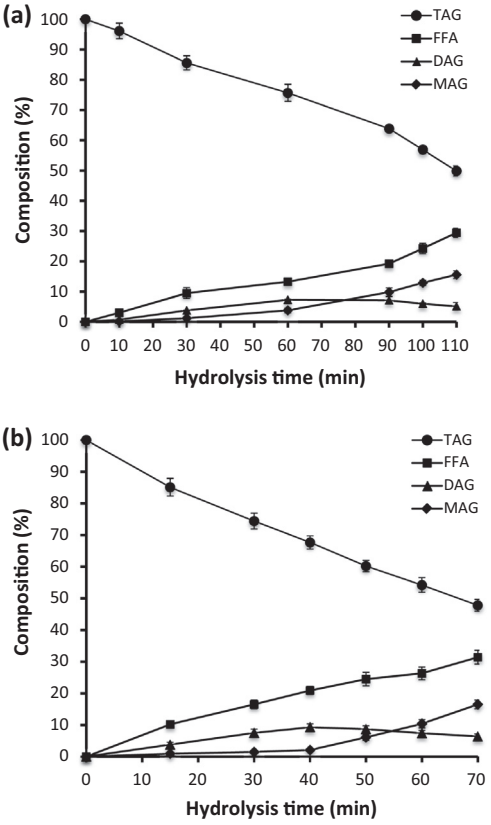

Fig. 1. Percentage hydrolysis and capillary chromatography (Iatroscan) profile of lipid classes from hydrolysed (a) anchovy oil and (b) seal oil using 10,000 units of porcine pancreatic lipase at 37 °C and pH 7.7. Values are expressed as mean ± standard deviation ($n = 3$).