Food Chemistry 173 (2015) 1030–1036

Contents lists available at ScienceDirect

Food Chemistry

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

A computational search for lipases that can preferentially hydrolyze long-chain omega-3 fatty acids from fish oil triacylglycerols

Md. Zahid Kamal [a,*], Colin J. Barrow [b], Nalam Madhusudhana Rao [a,*]

[a] Centre for Cellular and Molecular Biology, Council of Scientific and Industrial Research, Uppal Road, Hyderabad 500007, India
[b] Centre for Chemistry and Biotechnology, Deakin University, 75 Pigdons Road, Waurn Ponds, Victoria 3216, Australia

ARTICLE INFO

Article history:
Received 24 May 2014
Received in revised form 17 September 2014
Accepted 21 October 2014
Available online 29 October 2014

Keywords:
Omega-3 fatty acids
EPA
DHA
Lipase
Covalent docking

ABSTRACT

Consumption of long-chain omega-3 fatty acids is known to decrease the risk of major cardiovascular events. Lipases, a class of triacylglycerol hydrolases, have been extensively tested to concentrate omega-3 fatty acids from fish oils, under mild enzymatic conditions. However, no lipases with preference for omega-3 fatty acids selectivity have yet been discovered or developed. In this study we performed an exhaustive computational study of substrate–lipase interactions by docking, both covalent and non-covalent, for 38 lipases with a large number of structured triacylglycerols containing omega-3 fatty acids. We identified some lipases that have potential to preferentially hydrolyze omega-3 fatty acids from structured triacylglycerols. However omega-3 fatty acid preferences were found to be modest. Our study provides an explanation for absence of reports of lipases with omega-3 fatty acid hydrolyzing ability and suggests methods for developing these selective lipases.

1. Introduction

Omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) such as EPA (cis-5,8,11,14,17-eicosapentaenoic acid) and DHA (cis-4,7,10,13,16,19-docosahexaenoic acid) are required for human health (Bougnoux, Hajjaji, Maheo, Couet, & Chevalier, 2010; Demaison & Moreau, 2002; Gissi et al., 2008; Harris, 2009; Heird, 2001; Montgomery & Richardson, 2008; Riediger, Othman, Suh, & Moghadasian, 2009; Saremi & Arora, 2009; Shen, Peterson, Tatum, & Dunn, 2008). Several investigations have established the beneficial effects of the consumption of EPA and DHA, particularly for decreasing high blood pressure and inflammation, lowering the risks of atherosclerosis and ischaemic heart disease, and improving endothelial and vascular function (Demaison & Moreau, 2002; Gissi et al., 2008; Harris, 2009; Riediger et al., 2009; Saremi & Arora, 2009; Shen et al., 2008). The American heart association (AHA) recommended 1.0 and 2.4 g/day of EPA and DHA to individuals suffering from artery heart diseases and hypertriglyceridemia, respectively (Kris-Etherton, Harris, Appel, Association, & Nutrition, 2002), and several other organizations and regulatory groups have issued recommended consumption levels.

Humans cannot *de novo* synthesize omega-3 fatty acids hence they need to be obtained through diet. Fish oil is one of the best sources of omga-3 fatty acids. However, fish oil contains a maximum of about 30% EPA and DHA, hence concentrated forms are used in some nutritional supplements and as pharmaceutical ingredients (Kralovec, Wang, & Barrow, 2010). Omega-3 fatty acids are commonly concentrated from fish oil by complete hydrolysis followed by fractional distillation and/or by urea complexation. These processes are relatively damaging and lead to partial oxidation and polymerization of these oxidatively unstable omega-3 fatty acids (Shahidi & Wanasundara, 1998). Enzymatic processes offer an attractive alternative to conventional chemical approaches as they can be carried out under very mild conditions without the formation of undesirable byproducts. However, to be commercially useful the enzymatic processes need to be carried out with high selectivity so that high yields are obtained. Attempts to improve the selective hydrolysis of EPA and DHA from triacylglycerols of fish oil using lipases have met with limited success (Fernández-Lorente, Betancor, Carrascosa, & Guisán, 2011; Kralovec et al., 2010; Mbatia, Adlercreutz, Mulaa, & Mattiasson, 2010; Perez et al., 2011) and due to poor selectivity of lipases, the process is currently not commercially useful.

Each triacylglycerol molecule has one glycerol moiety esterified to three fatty acids. Natural fish oils are compositionally complex with variations both in the fatty acid and its position on the

* Corresponding authors at: W112, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, Andhra Pradesh, India. Tel.: +91 40 27192531; fax: +91 40 27160310 (Md.Z. Kamal). W105, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, Andhra Pradesh, India. Tel.: +91 40 27192504; fax: +91 40 27160310 (N.M. Rao).

E-mail addresses: zahid@ccmb.res.in (M.Z. Kamal), madhu@ccmb.res.in (N.M. Rao).

http://dx.doi.org/10.1016/j.foodchem.2014.10.124
0308-8146/

glyceride providing numerous potential structural combinations. Using ^{13}C NMR, gas chromatography and liquid chromatography-mass spectrometric methods both the positional information (Aursand, Jørgensen, & Grasdalen, 1995; Gunstone, 1991; Suárez et al., 2010) and fatty acid composition (Armenta, Scott, Burja, Radianingtyas, & Barrow, 2009; Saify et al., 2000) of fish oil triacylglycerols has been determined. A recent study demonstrated that *Thermomyces lanuginosus* lipase was partially selective for specific fatty acids rather than to their regio-position (Akanbi, Adcock, & Barrow, 2013). Computational studies have been promising in understanding substrate–enzyme interactions and in quantifying the interactions to identify substrate specificities. In the present study, we performed a computation search to find lipase(s) which have the potential to discriminate and preferentially hydrolyze DHA and/or EPA from natural fish oil triacylglycerols. We have chosen non-redundant lipases with known chemical structure from protein data banks and performed computational docking (both non-covalent and covalent docking) of various designed triacylglycerols differing in composition and placement of fatty acids on the glycerol.

2. Methods

Structural coordinates of non-redundant lipases (⩾10% variation in sequence) were obtained from a protein data bank (www.pdb.org). Lid regions of these lipases were identified through literature searching and deleted from coordinate files. These lidless lipases were used as the receptor template for docking experiments. Structural coordinate files for various triacylglycerol substrates were created using Chemdraw 3D Ultra (Mills, 2006). Autodock Tools (Morris et al., 2009) was used to prepare receptor (lipases) and ligand (triacylglycerols) files for docking experiments. In both files, non-polar hydrogen atoms were merged into covalently-bonded heavy atoms and Gasteiger charges were added. All torsions were allowed for triacylglycerols by accepting all rotatable bonds as such. Computational docking experiments were performed using Autodock Vina (Trott & Olson, 2010). Docking grid dimensions were set to 24 Å × 28 Å × 28 Å while default values were used for other parameters. Covalent docking experiments were performed using flexible side chain method (Morris et al., 2009).

3. Results and discussion

3.1. Docking

One hundred thirty lipase (EC 3.1.1.3) structures are available in the protein data bank (PDB). A large fraction of these structures are redundant as several lipase structures were reported multiple times, with variations such as crystallization under different conditions, with and without ligand, with mutations, or other often minor differences. For the present study we extracted the non-redundant lipase structures (⩾10% variation in sequence), which correspond to a total of 38 lipases. We have used PDB ids as the lipase identifier throughout this study. Complete identification of selected lipases along with their PDB ids are given in Table 1. Active sites of lipases are usually covered by a structural elements often referred to as a lid, which opens/displaces during catalysis making way for substrate entry into the active site. Scientific literature suggests that this is the only significant function of lids and they do not participate in substrate binding or specificity. Hence to make computational docking possible, which requires open active sites, we identified the lid regions of lipases and removed their structure coordinates from lipase PDB files.

Fish oils and other natural sources of omega-3 fatty acids are mixtures of triacylglycerols of varying chemical compositions.

Table 1
Description of lipases used in the study.

PDB ids	Lipase description
1BU8	Rat pancreatic lipase-related protein 2
1CRL	*Candida rugosa* lipase
1CUJ	*Fusarium solani* cutinase
1CVL	*Chromobacterium viscosum* lipase
1ETH	Porcine lipase
1EX9	*Pseudomonas aeruginosa* lipase
1F6W	Human bile salt activated lipase
1GPL	Chimeric pancreatic lipase-related protein 2 from guinea pig
1GZ7	Lipase 2 from *Candida rugosa*
1HLG	Human gastric lipase
1HPL	Horse pancreatic lipase
1I6W	*Bacillus subtilis* lipase
1K8Q	Dog gastric lipase
1LGY	Lipase II from *Rhizopus niveus*
1LLF	Cholesterol esterase from *Candida cylindracea*
1LPB	Pancreatic lipase from human
1QGE	*Pseudomonas glumae* lipase
1RP1	Dog pancreatic lipase-related protein 1
1TCA	Lipase B from *Candida antarctica*
1THG	Lipase from *Geotrichum candidum*
1TIB	Lipase from *Thermomyces lanuginosus*
1YS1	*Burkholderia cepacia* lipase
2BCE	Bovine pancreatic cholesterol esterase
2DSN	T1 lipase from *Geobacillus zalihae*
2FX5	*Pseudomonas mendocina* lipase
2HIH	*Staphylococcus hyicus* lipase
2ORY	M37 lipase from *Photobacterium lipolyticum*
2PPL	Human pancreatic lipase-related protein 1
2PVS	Human pancreatic lipase-related protein 2
2QUB	Extracellular lipase from *Serratia marcescens*
2Z8X	Extracellular lipase from *Pseudomonas sp.* MIS38
2ZYR	Lipase from alkalohyperthermophilic *Archaeoglobus fulgidus*
3G7N	Lipase from *Penicillium expansum*
3GUU	*Candida antarctica* lipase A
3ICV	Redesigned lipase B from *Candida antarctica*
3NGM	Lipase from *Gibberella zeae*
3O0D	*Yarrowia lipolytica* lipase Lip 2
3TGL	Lipase from *Rhizomucor miehei*

Careful analysis of chemical compositions of these oils suggests that triacylglycerol molecules usually contain one omega-3 fatty acid with the other two positions esterified to other kinds of fatty acids (Aursand et al., 1995; Suárez et al., 2010). Analysis of fish oils has indicated that EPA is usually statistically distributed amongst the three possible positions, whereas DHA is more abundant at the sn2 position (Aursand et al., 1995; Suárez et al., 2010). To reflect this natural region-positional information we selected triacylglycerols having EPA at sn1/sn3 and DHA at sn2 positions for computational docking. The remaining two positions in these triacylglycerols were filled with saturated C16 fatty acid, an abundant fatty acid in most fish oils (Armenta et al., 2009; Saify et al., 2000; Suárez et al., 2010). As a specificity control to these two substrates, we chose triacylglycerols having saturated fatty acids of C20 and C22 chain length in place of EPA at sn1/sn3 and DHA at sn2 positions, respectively. Proper structural coordinates of these substrate molecules were created using Chemdraw 3D Ultra (Mills, 2006). Computational docking experiments were performed using Autodock Vina (Trott & Olson, 2010). Fig. 1a shows the binding affinity of various triacylglycerol molecules with the lipases. Binding energy of lipases for these substrates varies in the range of −3.4 to −7.3 kcal/mol. It can also be observed that most of the lipases showed a narrow range of affinity for the substrates studied. That is, a lipase showing low affinity for one substrate also showed a similar level of affinity for other substrates, while lipases showing high affinity for a substrate did so for all the other substrates. For example, lipase 1YS1 showed high affinity in the narrow range of −6.5 to −6.8 kcal/mol for all four triacylglycerols. Similarly, lipase 1CUJ showed low affinity in the narrow range of −3.6 to −4.1 kcal/