Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1735 (2005) 50 – 58

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

The lipid droplet enzyme Tgl1p hydrolyzes both steryl esters and triglycerides in the yeast, *Saccharomyces cerevisiae*

Anita Jandrositz[a,1], Julia Petschnigg[a], Robert Zimmermann[a], Klaus Natter[a], Hubert Scholze[b], Albin Hermetter[b], Sepp D. Kohlwein[a], Regina Leber[a,*]

[a]*Institute of Molecular Biosciences, SFB Biomembrane Research Center, University of Graz, Schubertstr. 1, A8010 Graz, Austria*
[b]*Institute of Biochemistry, Graz University of Technology, A8010 Graz, Austria*

Received 3 February 2005; received in revised form 12 April 2005; accepted 21 April 2005
Available online 16 May 2005

Abstract

Based on sequence homology to mammalian acid lipases, yeast reading frame YKL140w was predicted to encode a triacylglycerol (TAG) lipase in yeast and was hence named as *TGL1*, triglyceride lipase 1. A deletion of *TGL1*, however, resulted in an increase of the cellular steryl ester content. Fluorescently labeled lipid analogs that become covalently linked to the enzyme active site upon catalysis were used to discriminate between the lipase and esterase activities of Tgl1p. Tgl1p preferred single-chain esterase inhibitors over lipase inhibitors in vitro. Under assay conditions optimal for acid lipases, Tgl1p exhibited steryl esterase activity only and lacked any triglyceride lipase activity. In contrast, at pH 7.4, Tgl1p also exhibited TAG lipase activity; however, steryl ester hydrolase activity was still predominant. Tgl1p localized exclusively to lipid droplets which are the intracellular storage compartment of steryl esters and triacylglycerols in the yeast *S. cerevisiae*. In a *tgl1* deletion mutant, the mobilization of steryl esters in vivo was delayed, but not abolished, suggesting the existence of additional enzymes involved in steryl ester mobilization.

Keywords: Lipid droplet; Triacylglycerol lipase; Acid lipase gene family; Fluorescent inhibitor; Steryl ester; Green fluorescent protein

1. Introduction

Triacylglycerols (TAG) and steryl esters are important, efficient and strongly hydrophobic storage forms for fatty acids and sterols in all types of eukaryotic cells. In mammals, depending on the cell type, neutral lipids are deposited in cytosolic lipid bodies and in plasma lipoproteins that are both assembled at the endoplasmic reticulum (ER) membrane. Whereas lipid bodies are eventually released from the ER membrane into the cytosol, the assembly of lipoproteins takes place in the lumen of the ER, followed by secretion to the cell exterior [1–3]. The seeds of many plant species contain lipid droplet-like structures, termed oleosomes, that store triacylglycerols as food reserves for germination and post-germinative growth of seedlings [4,5]. In the yeast *Saccharomyces cerevisiae*, lipid droplets (LD) are mainly composed of about equal amounts of steryl esters and triacylglycerols, which together make up some 98% of the LD mass [6]. All types of lipid bodies identified so far in mammalian cells, plants or yeast are characterized by a hydrophobic core formed by neutral lipids and a phospholipid monolayer delineating the surface of the lipid body [2]. In addition, lipid bodies contain a set of proteins that may be associated with or embedded in the surface phospholipid layer [2,7–11].

During cellular proliferation, i.e. upon increased cellular demand for free sterols or fatty acids, neutral lipids become

Abbreviations: LD, lipid droplet; ER, endoplasmic reticulum; TAG, triacylglycerol; FFA, free fatty acid; HLAL, human lysosomal acid lipase; PerGP, 1-*O*-hexadecyl-2-*O*-perylenedodecyl-*sn*-glycero-3-phosphonic acid–(*n*-hexyl)-*p*-nitrophenyl-ester; PerMBP, *O*-perylenebutylmethylphosphonic-acid–*p*-nitrophenyl ester; eGFP, enhanced green fluorescent protein; DIC, differential interference contrast

* Corresponding author. Tel.: +43 316 380 5486; fax: +43 316 380 9857.
E-mail address: regina.leber@uni-graz.at (R. Leber).
[1] Present address: Lifecord Inc., A8010 Graz, Austria.

1388-1981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2005.04.005

mobilized from lipid droplets by the action of lipolytic enzymes. Steryl esters and triacylglycerols stored in lipid droplets thus provide an important source of lipid intermediates for membrane biogenesis [12,13]. Recently, Tgl3p was identified as a triacylglycerol lipase associated with lipid droplets in yeast, and mutants deficient in this enzyme show a delayed mobilization of triacylglycerols under conditions of fatty acid starvation [14]. The characterization of lipid droplet proteins by mass spectrometry identified Tgl1p, which was predicted to be a triacylglycerol lipase, based on sequence similarity with human gastric lipase and rat lingual lipase, two members of the acid lipase gene family [9,15]. Thus, the picture emerges that LD are not only sites of triacylglycerol and steryl ester storage but also contain enzymes for neutral lipid mobilization.

A deletion of *TGL1* led to an increased cellular level of steryl esters [9], indicating that Tgl1p which resides on LD might act on steryl esters. Very recently, Yeh2p, encoded by YLR020c, was shown to function as a steryl ester hydrolase at the plasma membrane [16]. This particular localization of Yeh2p is puzzling, in view of the fact that the source of substrate is exclusively present in lipid droplets. Moreover, a substantial amount of steryl esters consists of intermediates of ergosterol [7], which require conversion to the end-product prior to the incorporation of free ergosterol into the plasma membrane. Enzymes catalyzing sterol biosynthesis are, however, mainly located in the endoplasmic reticulum (ER) [7,17].

The findings that a deletion of *TGL1*, which was predicted to encode a putative triacylglycerol lipase, results in a two-fold increase of the steryl ester to phospholipid ratio, whereas the triacylglycerol to phospholipid ratio remained unchanged in the *tgl1* mutant [9], led us to investigate the substrate specificity of Tgl1p. In order to discriminate between a lipase or an esterase activity of Tgl1p, we made use of fluorescent lipid analogs that become covalently linked to the enzyme active site upon catalysis [18]. This approach revealed that Tgl1p prefers single-chain esterase inhibitors over lipase inhibitors in vitro. Furthermore, steryl ester hydrolase activity was increased in cell extracts from strains overexpressing *TGL1*, and this activity was specifically inhibited by antibodies directed against Tgl1p. The hydrolytic activity of Tgl1p was, however, not restricted to steryl esters, it also showed minor activity against triolein at neutral pH. Under conditions optimal for acid lipases, Tgl1p revealed steryl esterase activity only. Tgl1p localized exclusively to lipid droplets, the site of steryl ester and triacylglycerol storage, in yeast.

2. Materials and methods

2.1. Strains and media

The yeast strains used in this study were CH1305 (*MATa ade2 ade3 leu2 ura3 lys2*) [19], BY4742 (*MATα his3 leu2 lys2 ura3*, Euroscarf, Germany) and *tgl1 (MATa his3 leu2 met 15 ura3*YKL140w::kanMX4, Euroscarf, Germany). A yeast strain expressing a full-length, chromosomally GFP-tagged Tgl1p fusion protein was obtained from Open-Biosystems [20]. The protease-deficient strain cl3-ABYS-86 (*MATα pra1-1 prb1-1 prc1-1 cps1-3 ura3Δ5 leu2-3,112 his3*) was kindly provided by D.H. Wolf (University of Stuttgart, Germany). The *E. coli* strain TOP10F′ {[proAB, laqI^q, lacZΔM15, Tn10(Tet^r)], mcrAΔ(mrr-hsdRMS-mcrBC), phi80ΔlacZM15, ΔlacX74, deoR, recA1, ara-D139Δ(ara, leu), 7697galU, galK, λ-rps(streptomycin^r), endA1, nupG} was used for plasmid amplification. Ampicillin-resistant *E. coli* transformants were selected on LBA (5 g/l yeast extract, 10 g/l tryptone, 5 g/l NaCl, and 100 mg/l ampicillin (Amresco)). Yeast strains were grown in YPD (10 g/l yeast extract (USB), 20 g/l peptone (USB) and 20 g/l glucose (Merck)), YPRaf (10 g/l yeast extract (USB), 20 g/l peptone (USB) and 10 g/l raffinose (Acros Organics)) or YPRafGal (YPRaf plus 10 g/l galactose (Sigma)). Geneticin resistance was scored on YPD plates containing 200 mg/l geneticin/G418 (Calbiochem). Yeast transformants carrying plasmids were grown on a uracil-free minimal medium (6.7 g/l YNB (USB), 10 g/l glucose, supplemented with the respective amino acids). The expression of GST–Tgl1p was induced by the addition of 0.5 mM $CuSO_4$ to the medium.

2.2. Construction of the plasmid pAJ-GST-TGL1

The *TGL1* gene was amplified by PCR from wild type CH1305 genomic DNA using a proofreading Pwo DNA polymerase from Roche and the primers TGL1(GST, *Bam*HI) and TGL1(GST, *Not*I) (Table 1). Plasmid pAJ-GST-TGL1 was generated by the insertion of the *TGL1* gene into the *Bam*HI/*Not*I sites of the yeast GST-fusion expression vector pYEX-P, generously provided by A.

Table 1
Primers used in this study

Primer	Sequence (5′→3′)
TGL1-GAL	GAACAAGGAAAGAAGAAAGAAAACAATTCGAACAAAACTTTATTATTCTAGCACTATTTTATCGATGAATTCGAGCTCG
GAL-TGL1	TACCAAGACGACTATAATGTAATCTGTTATCGATAATCTGCCTAAAAAGGGGAAGTACATCGTACGCTGCAGGTCGAC
U2	GGTTGTTTATGTTCGGATGTG
TGL1-rev	TTCATGGATAGTTGGAGCTG
TGL1 (GST, *Bam*HI)	ATGGATCCATGTACTTCCCCTTTTTAGGCAG
TGL1 (GST, *Not*I)	ATGCGGCCGCTCATTCTTTATTTAGAGCATCCAGC