Food Chemistry 115 (2009) 488–494

Contents lists available at ScienceDirect

Food Chemistry

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

In vitro comparisons between *Carica papaya* and pancreatic lipases during test meal lipolysis: Potential use of CPL in enzyme replacement therapy

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ARTICLE INFO

Article history:
Received 17 July 2008
Received in revised form 7 December 2008
Accepted 9 December 2008

Keywords:
Lipase
Carica papaya latex
Test meal
Triacylglycerols
Replacement therapy

ABSTRACT

High levels of lipase activity are known to occur in *Carica papaya* latex, and this activity is being used in some biotechnological applications. The lipolytic activity of *C. papaya* lipase (CPL) on dietary triacylglycerols (TAG) has not yet been studied. Hence, the aim of this study was to characterise the specific activity of CPL on dietary TAG present in a crude preparation. Also, we have determined its stability during the lipolysis of a test meal at various pH values mimicking those occurring in the gastro-intestinal tract, with or without bile, and have compared these properties with those of porcine pancreatic extract (PPE) and human pancreatic lipase (HPL). CPL showed maximum stability at pH 6.0, both with and without bile. Some residual activity was still observed at pH 2 (20%), whereas the pancreatic lipases tested were immediately completely inactivated at this pH. In the absence of bile, the highest specific activities were measured at pH 6 in the case of CPL, PPE and HPL. Adding bile slightly decreased the CPL activity in the 4–6 pH range, thus shifting the optimum CPL activity to pH 7, where the presence of bile had no effect. Lipolysis levels decreased with the pH, but CPL was still more active than PPE at pH 5 on a relative basis. These results suggest that CPL might be a promising candidate for use as a therapeutic tool on patients with pancreatic exocrine insufficiency.

1. Introduction

Gastric and pancreatic lipases are the main enzymes involved in the digestion of dietary triacylglycerols (TAG) in the digestive tract (Carrière, Barrowman, Verger, & Laugier, 1993; Lengsfeld, Titgemeyer, Faller, & Hensel, 2004). Both of these lipases have been biochemically characterised in detail (Roussel et al., 2002) and their respective contributions during the digestion of a test meal estimated in healthy human volunteers and patients with severe exocrine pancreatic insufficiency (EPI) (Carrière et al., 2005).

Exocrine pancreatic insufficiency occurs when less than 10% of the normal amount of digestive enzymes is secreted by the pancreas (Aloulou et al., 2008). Lack of lipolytic enzymes leads to the loss of fat via the stools, which may lead to malnutrition. Main symptoms of exocrine pancreatic insufficiency include steatorrhea, creatorreah, hypoproteinemia and fat-soluble vitamin and essential fatty acid deficiency. Oral pancreatic enzyme supplements are widely used as the first-line approach to maldigestion secondary to exocrine pancreatic insufficiency, whatever the aetiology (Breithaupt, Alpmann, & Carrière, 2007). To deal with the problems associated with acid-mediated inactivation of pancreatic lipase, especially in patients with EPI who also show low pH values in the small intestine (Carrière & Laugier, 2005), pharmacological enteric-coated pancreatic enzyme formulations have been developed, which release the enzyme whenever the pH exceededs a threshold value of 5.0–5.4 (Dutta, Rubin, & Harvey, 1983). Enteric-coated mini-microspheres, which are emptied from the stomach together with nutrients, release the active pancreatic enzymes they contain within the proximal intestine (Aloulou et al., 2008). Pancreatic enzyme supplementation is fundamental to the management of these conditions, but suitable monitoring methods are lacking and this therapeutic area is generally regarded as being unsatisfactory (Layer, Keller, & Lankisch, 2001). Porcine pancreatic extract (PPE) is used in current standard treatments, usually in the form of enteric-coated microsphere preparations (Layer et al., 2001). The Food and Drug Administration has recently decreed, however, that all pancreatic enzyme products will require an approved new drug application, because these products of animal origin are associated with the risk of viral transmission, and differences in their pharmaceutical quality have been observed. These animal products may therefore be replaced in the near future by enzymes produced using genetic engineering methods or purified from plants.

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doi:10.1016/j.foodchem.2008.12.043

When studying novel lipases for enzyme replacement therapy purposes, it is particularly important to determine the combined effects of bile and pH (Aloulou, Puccinelli, De Caro, Leblond, & Carrière, 2007) on lipase activity because the intraluminal digestion of fat occurs under conditions where bile salts as well as other amphiphilic compounds and large pH variations are liable to affect the lipase-catalysed interfacial process of lipolysis. This is true in the case of the endogenous human gastric and pancreatic lipases as well as the various lipases that might potentially be used for enzyme replacement therapy purposes on patients with EPI (Aloulou et al., 2008).

Most of the findings available so far in the field of lipase research and development focus on microbial and animal lipases (Cambon et al., 2008; Mukherjee & Kiewitt, 1996). Plant lipases are interesting enzymes, however, that might combine the advantages of competitive prices and a large spectrum of specificities and stabilities (Villeneuve et al., 2007). During the last few decades, considerable attention has therefore focused on these enzymes (Lee, Xia, & Zhang, 2008). Various plant sources of lipases and suitable techniques for isolating and purifying them have been well documented (Fiorillo, Palocci, Soro, & Pasqua, 2007; Giordani, Moulin, & Verger, 1991; Palocci et al., 2007; Suzuki, Honda, & Mukasa, 2004). Lipases belonging to plant families such as *Euphorbiaceae* (Giordani et al., 1991; Moulin, Teissere, & Bernard, 1994), *Asclepiadaceae* (Giordani et al., 1991), *Brassicaceae* (Hills & Mukherjee, 1990) and *Caricaceae* (Giordani et al., 1991) have been described as providing useful biocatalysts for several applications.

High rates of lipase activity were found to occur in the latex of *Carica papaya* (Giordani et al., 1991). This latex is used to prepare well-known commercially available enzyme preparations containing papain and chymopapain, and these enzymes have also been employed for many years in the food (as a meat tenderiser, for example), beverage and pharmaceutical industries (Leipner & Saller, 2000). These proteinases are usually extracted as water-soluble proteins from the latex (Azarkan, El Moussaoui, Van Wuytswinkel, Dehon, & Looze, 2003). On the other hand, since *C. papaya* lipase (CPL) remains tightly attached to the solid particles present in the *C. papaya* latex, it is insoluble in water (Giordani et al., 1991). Due to this feature, it has traditionally been regarded as a "naturally immobilised" biocatalyst (Leipner & saller, 2000; El Moussaoui et al., 2001). A dry powder containing lipase activity can be obtained after washing the latex particles with water and centrifuging them.

In the present study, the specific activities and the stability of a crude CPL preparation were measured *in vitro* during the lipolysis of a test meal. Various conditions (various pH levels, the presence versus absence of bile) were tested in order to predict the possible action of CPL *in vivo*. For the sake of comparison, similar experiments were carried out using porcine pancreatic extract (PPE) and human pancreatic lipase (HPL).

2. Materials and methods

2.1. Test meals and lipolysis experiments

The test meal was prepared by mixing 80 g string beans, 90 g grilled beef meat, 70 g French fries, 10 g butter, 20 g sunflower oil: all these ingredients were purchased in local grocery stores (Marseille, France). All the solid components were first minced using a mincer with 2-mm apertures and then mixed with the oil in order to obtain a homogeneous paste. In each assay, 5.8 g of this paste were mixed with 12.2 ml water, thus mimicking a meal diluted with gastric juice, as occurs in the stomach at half gastric emptying time (dilution ratio 5/1, v/v) (Carrière et al., 2000). To improve the reproducibility of the procedure, water was added just before the beginning of the assay so that the meal components would not separate. When experiments were performed with human bile, the meal paste (5.8 g) was diluted with 1 ml human bile and 11.2 ml water.

The amounts of triacylglycerols (TAG) present in the assay mixture were found to be 0.9 g after extracting the total lipids with chloroform/methanol (2:1, v/v) and quantifying the TAG using thin-layer chromatography coupled to flame ionisation detection (TLC-FID) methods (see Section 2.4).

Each experiment was performed in a 50-ml thermostated (37 °C) vessel equipped with a pH electrode and a 1-cm magnetic rod rotating at a speed of 1000 rpm. At time 0, the lipase sample to be tested was added and 200 μl of the reaction mixture were immediately sampled to measure the lipase activity using a pH-stat. Similar sampling and assay procedures were performed at times 0, 5, 10, 15, 30 and 60 min to determine the stability of the enzyme by measuring its activity on tributyrin. One-millilitre of each sample was also collected at times 0, 5, 10, 15, 30 and 60 min and used for total lipid extraction and lipolysis product analysis.

2.2. Lipase activity measurements

A pH-stat equipment (Metrohm 718 Stat Titrino, Zofingen, Switzerland) was used to measure the lipase activities potentiometrically in a thermostated reaction vessel (37 °C) containing a mechanically stirred emulsion of 0.5 ml tributyrin (Fluka, Paris, France) in 14.5 ml of an assay solution specifically prepared for each lipase (Cherif, Frikha, Gargouri, & Miled, 2008).

CPL activity was measured at pH 9.0 in 150 mM NaCl, 5 mM $CaCl_2$ and 2 mM Tris–HCl. Pancreatic lipase activity was measured at pH 8, 150 mM NaCl, 1.4 mM $CaCl_2$, 4 mM NaTDC, 0.28 mM Tris–HCl in the presence of excess colipase.

2.3. Enzymes

2.3.1. Carica papaya lipase (CPL)

A crude CPL preparation was obtained as a waste product of industrially produced papain. The latex from *C. papaya* is usually dried and roughly crushed in order to obtain a crude powder called P_3. A non-water-soluble cake containing lipase activity was then obtained after solubilisation of the latex in water in the presence of additives, before performing various filtration steps (Baines & Brocklehurst, 1979). This cake was further washed to completely remove all the water-soluble part of the latex and then lyophilised to obtain a powder containing >95% dry matter. The complete process lead to the mass recovery of about 35% (w/w) of cake compared with the crude latex initially used in the process, and most of the lipolytic activity initially present in the latex was recovered in this *C. papaya* lipase preparation having a specific activity of 2000 U/g, using tributyrin as a substrate. This material will be referred to here as *C. papaya* lipase (CPL). Fifty milligrams of CPL were used for each assay.

2.3.2. Porcine pancreatic extract (PPE)

The contents of a Creon 25000® capsule (512 mg of PPE mixed with excipients) were dissolved in 15 ml of a 100 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer at pH 7. The pH had to be kept constant during the whole dissolution phase by gradually adding small volumes of a 0.1 M NaOH solution. The lipase activity of the PPE solution measured using a tributyrin substrate was found to be 3520 U/ml. Since the specific activity of pure porcine pancreatic lipase (PPL) is 8000 U/mg under these conditions, we calculated that the mean PPL concentration in the PPE solution was 0.44 mg/ml. Since the PPE + excipient mass concentration in

the solution was 34 mg/ml, the PPE + excipient mixture therefore contained 1.3% (w/w) of PPL. A volume of 2.3 ml PPE solution (i.e., 78 mg PPE + excipient or 1 mg PPL) was used for each test meal lipolysis assay.

2.3.3. Human pancreatic lipase (HPL)

Recombinant human pancreatic lipase (rHPL) was produced in *Pichia pastoris* and purified from the culture media at our laboratory, as described by Belle et al. (2007). The specific activity of the purified rHPL was found to be 8000 U/mg using the standard HP assay (see lipase activity measurements). A stock solution of rHPL at a concentration of 1 mg/ml was prepared and 1 ml of this solution (i.e. 1 mg HPL) was used in each assay.

2.4. Extraction and quantitative analysis of lipolysis products

Lipid extraction was performed immediately after sampling the reaction mixture, using Folch's procedure (Folch, Lees, & Sloane-Stanley, 1975). One-millilitre of the reaction mixture was sampled and poured into a 15-ml glass tube with a screw cap, filled with 200 μl 0.1 M HCl and 5 ml chloroform/methanol (2:1, v/v) and shaken vigorously. After phase separation, the lower organic phase was collected using a Pasteur pipette and transferred to a 15-ml test tube in which it was dried over anhydrous $MgSO_4$. Once the $MgSO_4$ had precipitated, 1–2 ml of the clear lipid extract was transferred to a 1.8-ml vial with a screw cap and kept at −20 °C until the analyses were performed.

Quantitative analysis of triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG) and free fatty acid (FFA) was performed by thin-layer chromatography coupled to flame ionisation detection (TLC-FID technique) methods with a an Iatroscan MK5 equipment (Iatron Laboratories, Tokyo, Japan) (Alasalvar et al., 2003; Carrière et al., 2005; Miraliakbari & Shahidi, 2008). To separate any neutral lipids, 1 μl of each lipid extract was spotted onto a silia-coated quartz rod (0.9 mm in diameter, ChromarodTM SIII, Iatron Laboratories) and sample migration was performed with heptane/diethyl ether/acetic acid (55:45:1, v/v/v).

After approximately 20 min, the chromarod holder (carrying 10 chromarods) was removed from the TLC tank and the chromarods were dried at 150 °C for 15 min. (Rod dryer TK8, Iatron Laboratories, Tokyo, Japan). The chromarod holder was then transferred into the Iatroscan MK5 and each chromarod was scanned by the FID to detect and quantify the compounds separated on silica. Since the ionisation current generated by burning a compound in the hydrogen flame is proportional to the mass of this compound, known amounts of standard compounds (0.1–10 μg triolein, [1,2- and 1,3-] diolein, monoolein, and oleic acid) were used to calibrate the FID mass detection procedure. A calibration curve (peak area vs. mass) was drawn up for each class of compound analysed and used to quantify the lipid masses in the various samples analysed (Carrière et al., 2000). The mass detection data were converted into moles using the following average molar masses calculated on the basis of the fatty acid composition of the test meal triacylglycerols: 821.9 g/mol for TAG, 578.6 g/mol for DAG, 335.4 g/mol for MAG and 261.3 g/mol for FFA.

2.5. Expression of lipase specific activities on test meal TAG

Specific activities (SA) were either expressed in terms of units (1 U = 1 μmol of FFA released per minute) per mg of purified enzyme (rHPL, PPL) or units per g of crude powder (PPE, CPL). In optimised lipase assays (with a pH-stat), the specific activity usually remains constant for several minutes. Under the present experimental conditions, where the amount of substrate in the test meal was a limiting factor and the enzyme was presenting large excess, the lipolysis rate was expected to decrease rapidly, and the specific activity therefore did not remain constant with time (Carrière et al., 2000). SA was therefore arbitrarily estimated during the first 5-min incubation period.

2.6. Calculation of lipolysis levels

During the hydrolytic process, one molecule of TAG can release a maximum of 3 molecules of FFA. The rate of hydrolysis (or lipolysis) is usually defined as the percentage of the acyl chains (in mmoles) released from the meal TAG_0 (in mmoles):

$$\text{Hydrolysis } (\%) = \frac{100 \cdot \text{FFA}}{3 \cdot \text{TAG}_0}$$

The complete absorption of fat requires only the conversion of meal TAG into MAG, which corresponds to the release of 2 FFAs from 1 TAG molecule, i.e. a 66.6% level of lipolysis, according to the above definition. In the present study, we also used a second definition for the rate of lipolysis, which directly reflects the potential fat absorption rate during the enzymatic hydrolysis process. The lipolysis level was expressed in this case as the percentage of the total meal TAG acyl chains converted into "intestinally absorbable" acyl chains, i.e. FFA and MAG. It is defined by the following equation, in which TAG, DAG, MAG and FFA are the amounts (in mmoles) of residual TAG and lipolysis products recovered at a given time during the hydrolytic process:

$$\text{Hydrolysis } (\%) = \frac{100 \cdot \text{FFA}}{3 \cdot \text{TAG} + 2 \cdot \text{DAG} + \text{MAG} + \text{FFA}}$$

According to this definition, 100% lipolysis corresponds to the conversion of 1 TAG molecule into 1 MAG and 2 FFA molecules.

3. Results

The choice of experimental conditions used to simulate meal fat digestion *in vitro* was based on previous *in vivo* and *in vitro* studies, in which pancreatic and gastric lipase were compared (Carrière et al., 2000). Test meal dilution, bile concentration and pH values were based on data recorded during *in vivo* test meal digestion experiments (Carrière et al., 1993, 2000, 2001).

3.1. Stability of CPL, PPE and HPL in test meals with and without bile

Once the lipases had been mixed with the test meal, samples were collected at various intervals to measure the residual lipase activity on tributyrin. In the absence of bile, the stability of CPL was found to be optimum at pH 6, where 82% residual activity was measured after 5 min of incubation. The stability of CPL was also found to decrease with lowering pH and increasing time (Fig. 1a). At pH 2, the residual activity was found to be less than 20% after 5 min. Stability at pH 7.0 was also lower than that measured at pH 6. In the presence of bile (Fig. 1b), a similar pH stability of CPL was observed with the greatest stability found at pH 6 and a lower stability of CPL at lower pH values and at pH 7. These results indicate that bile does not have any effects worth noting on the stability of CPL.

Using porcine pancreatic extract (PPE) (Fig. 1c), the greatest PPE stability was observed at pH 6–7, and no decrease in the rates of activity occurred with time. A loss of activity was observed below pH 6, and PPE inactivation accelerated at pH 4 and started levelling off at pH 3. The activity was completely lost in this case at pH 2. Bile did not have any significant effect on PPE stability (Fig. 1d).

The recombinant human pancreatic lipase (HPL) stability profiles were found to differ significantly from those of PPE at pH values above 4 (Fig. 1e), where this lipase was less stable than PPE. The maximum stability of the HPL activity was observed at pH 7,

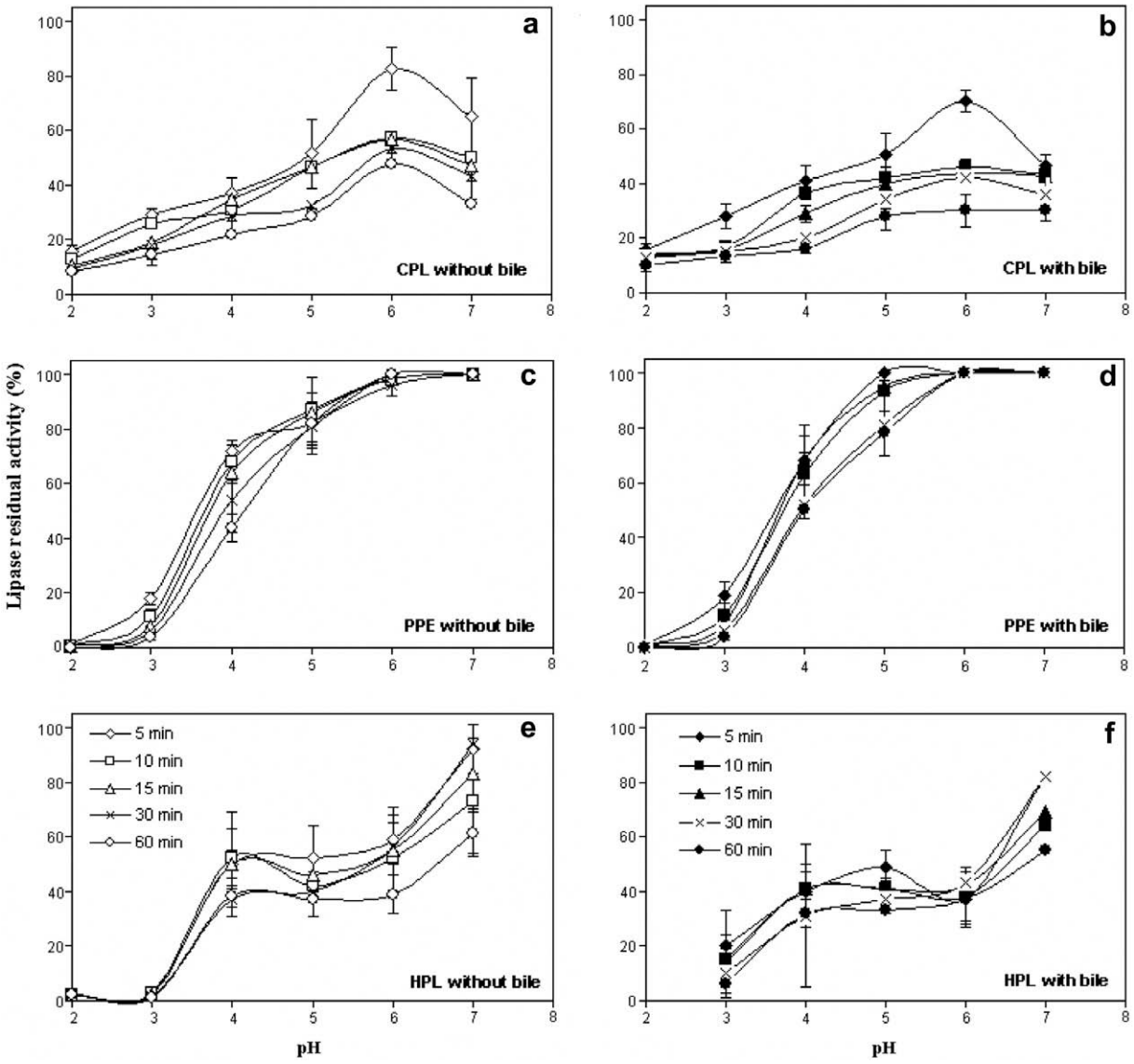

Fig. 1. Stability of lipase in the test meal, in the absence (white symbols) and presence (black symbols) of bile. (a and b) CPL, (c and d) PPE and (e and f) HPL.

and the mean residual activity after 30 min was greater than 80%. In the absence of bile, HPL was also completely inactivated at pH 2 and 3, whereas some activity was recovered with PPE at pH 3. Bile significantly affected HPL stability only at pH 3 (Fig. 1f), where some activity (20%) was recovered after 5 min.

3.2. Apparent specific activities of CPL, PPE and HPL on test-meal-triacylglycerols with and without bile

Although the lipases tested did not appear to be fully stable during the first few minutes of incubation with the test meal, the apparent specific activities on test meal TAGs were assessed from the FFA released during the first 5 min of incubation, except at pH 2 in the case of HPL and PPE, where these lipases were completely inactivated within a few seconds. Specific activities were found to decrease at longer incubation times (data not shown), probably due to the occurrence of enzyme inactivation, as well as to inhibition by lipolysis products and changes in the oil–water interface properties (Aloulou et al., 2006).

In the absence of bile, the highest specific activities were observed at pH 6 with all three lipases (Fig. 2). The specific activity of HPL was 31 ± 9 U/mg, which is comparable to the specific activities recorded in previous studies with HPL (15–43 U/mg) and human gastric lipase HGL (32–34 U/mg) using test meal TAGs as substrates (Carrière et al., 2000). The specific activities of CPL and PPE at pH 6 were 55.5 ± 4 and 526 ± 78 U/g, respectively. The specific activity of PPE was used to estimate that of the PPL (39 ± 3 U/mg) based on the known mass proportion (1.3%, w/w) of PPL in PPE. In all the cases tested, the specific activity decreased with decreasing pH, and it was also lower at pH 7 than at pH 6. Adding bile resulted in a pH shift corresponding to optimum specific activities at pH 6–7 in the case of CPL and PPE/PPL. This was in fact due to a decrease in the activity occurring at pH 6 rather than to an increase at pH 7. This is consistent with the increase in the stability of HPL observed at pH 3 in presence of bile. Control experiments with the test-meal incubated at pH 2 with and without bile and water instead of enzyme did not show the occurrence of any significant lipolysis. The differences between the profiles of HPL and PPE

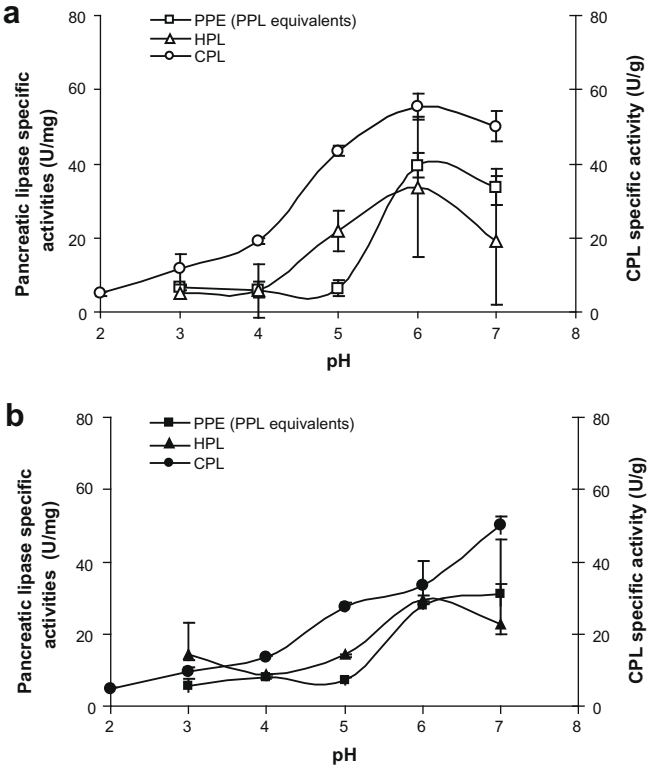

Fig. 2. Apparent specific activities of CPL, PPE (PPL equivalents) and HPL measured after 5-min incubation periods with the test meal, in the absence (Panel a) and presence (Panel b) of bile.

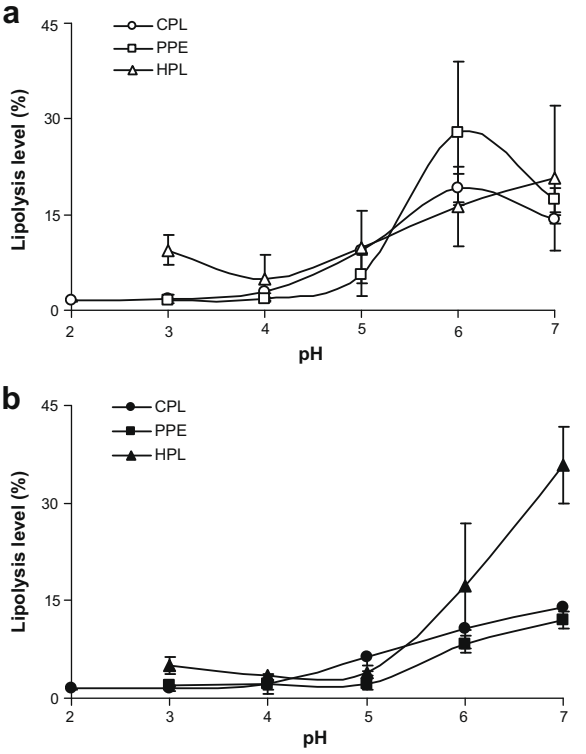

Fig. 3. Lipolysis levels obtained at various pH values after 60 min of incubation in the absence (Panel a) and presence (Panel b) of bile.

probably resulted from the fact that PPE is a mixture of several lipolytic enzymes.

3.3. Lipolysis levels of test meal triacylglycerols

Since the stabilities and therefore the specific activities of the lipases with time were variable, we also determined the lipolysis levels at various times with each lipase. Lipolysis levels increased during the first few minutes of incubation, but slowed down after 30 min, probably because of the concomitant decrease in the lipase activity and stability. Maximum lipolysis levels were observed at pH 6 with CPL (19.2%) and PPE (28%) after 60 min in the absence of bile (Fig. 3a). The presence of bile in the reaction mixture (Fig. 3b) decreased the lipolysis levels at pH 6, giving an optimum rate of lipolysis at pH 7. These results are in line with the findings obtained on the specific activity (Fig. 2) and suggest that PPE might not be saturated in colipase, the cofactor required to maintain the activity of pancreatic lipase in the presence of bile and induce interactions with lipase in a one to one molar ratio. With CPL, the lipolysis levels detected at pH 5 and 7 were not significantly decreased in the presence of bile, which suggests that this lipase is fairly resistant to inhibition by bile salts. At acidic pH values (ranging from 3 to 5), the specific activity and lipolysis levels of PPE were found to be very low, in good agreement with the data available in the literature on pancreatic lipase activity at various pH levels (Borgstrom, 1975).

The maximum rates of lipolysis occurring with HPL were always recorded at pH 7, but these rates were higher in the presence (35.9 ± 5.9%) than in the absence of bile (20.7 ± 11.3%). Bile therefore had a greater effect on HPL (Fig. 3b) than on the other lipases tested, probably because these experiments were performed with excess colipase in order to ensure maximum binding of the enzyme at the oil–water interface. Colipase is generally assumed to have significant positive effects on lipolysis level in the presence of bile (Freie, Ferrato, Carrière, & Lowe, 2006). At pH 5 in the presence of bile, the lipolysis level was higher with CPL (6.4% ± 1.6) than with HPL (3.9% ± 0.3) and PPE (2.2% ± 0.9). These values were also expressed as percentages of the maximum lipolysis level recorded at pH 7 with each enzyme: 45.5% with CPL, 18.5% with PPE and 10.8% with HPL. On a relative basis, CPL is therefore more active at pH 5 than PPE.

4. Discussion

The results of this study showed the existence of contrasting patterns of stability between CPL versus and PPE: the latter lipase was much more stable in the 5–7 pH range (Fig. 1a and b). The stability of CPL was higher, however, than that of PPE at pH 2–3, and some lipolysis of the test meal TAGs was observed at pH 2 (Fig. 3). The pH stability range of pure HPL was also found to be much lower than that of PPE. The lipase from PPE was probably stabilised by other components of the pancreatic extract, or the activity mea-

Table 1
Maximum specific activities of CPL, PPE and purified human gastric (HGL) and pancreatic (HPL) lipases on various triacylglycerols (TAG) and meal TAG.

	Long chain TAG	Tributyrin	Solid meal TAG
CPL (U/g)	300	2000	55
PPE (U/g)	38,000	103,000	526
Ratio	126	51.5	9.5
HGL (U/mg)	600[a]	1000[a]	32[b]
HPL (U/mg)	3000[c]	8000[c]	31[b]
Ratio	5	8	1.09

[a] From Gargouri et al. (1986).
[b] From Carrière et al. (2005).
[c] From Lengsfeld et al. (2004).

sured might have resulted from activity other than that of classical pancreatic lipase. Systematic studies on the effects of pH have been performed with various pancreatic and gastric lipases (Borgstrom & Erlanson, 1973; Carrière et al., 1991), but this is the first time the pH stability of CPL has been studied. Since CPL is more stable than pancreatic lipases at pH 2, this lipase might be a promising candidate for enzyme replacement therapy, since the pH values in the gastro-intestinal tract of patients with pancreatic insufficiency are usually lower than in healthy subject (Hills & Mukherjee, 1990).

The specific activities of CPL, PPE and HPL determined *in vitro* during the present study were much lower than those usually measured under optimised assay conditions (Table 1). For instance, the specific activities of PPE and CPL recorded *in vitro* on solid meal TAGs were 195- and 36-fold lower than the PPE and CPL activities observed on tributyrin, respectively. It has been reported, however, that the specific activities measured with meal TAGs are similar to those of the lipases *in vivo* (Carrière et al., 2000). When a solid test meal was used, the specific activity of PPE (526 U/g) was found to be 9-fold higher than that of CPL (55 U/mg). Under optimised *in vitro* assay conditions, the specific activity of PPE on tributyrin (103,000 U/g) was found to be 51-fold higher than that of CPL (2000 U/g). Similar results were reported by Carrière et al. (2000) upon comparing the behaviour of human pancreatic and gastric lipases on test meal TAGs.

Considerable agreement was observed here between the lipase stability, specific activity and lipolysis levels recorded in this study. The specific activity and the stability of CPL at pH values equal to or lower than 6.0 suggest that this enzyme might provide a useful therapeutic tool for improving the process of lipolysis in patients with pancreatic insufficiency.

In conclusion, CPL has several biochemical properties enabling it to act in the gastro-intestinal tract like mammalian digestive lipases: (i) its activity on long-chain TAGs reaches an optimum at pH 6.0 in the presence of bile, (ii) it is only weakly inhibited by bile salts, (iii) it shows a similar pattern of regioselectivity to that of human pancreatic lipase, generating 2-MAG and FFA (Cambon et al., 2008), the lipolysis products absorbed at the intestinal level, and (iv) it shows significant levels of stability and activity at low pH values at a temperature of 37 °C. CPL therefore seems to be tailored to act optimally under the physiological conditions pertaining in the gastro-intestinal tract. Its sensitivity to digestive proteases still needs to be tested, however. The relative activity of CPL observed at pH 4–5 is high in comparison with that of PPE. This study clearly shows that CPL is a true lipase acting on long-chain TAGs of the kind usually present in the human diet. It is worth nothing that CPL has always been described as a lipase with a preference for medium chain triacylglycerols, and this might explain why its potential use in enzyme replacement therapy has not investigated so far. Due to its biochemical properties, CPL might be a suitable candidate for use as a therapeutic tool on patients with pancreatic exocrine insufficiency. CPL is also a good candidate for use in biotechnological applications on the industrial scale (e.g. in the production of food ingredients and triacylglycerol transesterification reactions) (Tsai, Chen, Yang, Ng, & Chen, 2006). It will be most interesting to follow future developments in clinical research as well as the future biotechnological applications of this highly promising plant enzyme.

Acknowledgements

The authors are grateful to Professor Abdelkarim Abousalham from Claude Bernard University (Université de Lyon I) and Doctor Robert Verger from EIPL-CNRS for fruitful discussions. The authors thank Doctor Gabrielle Beamier-Gallon for her technical assistance during the lipolysis of test meals by pancreatic lipase. We also thank Mr Peter Van Cauwenberghe, the Managing Director of Biohainaut for his continuous support, and Christophe Collet (CIRAD) and Pierre Michaud (OSEO ANVAR, France) for their help in setting up this project. English revision by Dr. Jessica Blanc is acknowledged. This study was performed in the framework of the European EUREKA LIPLANT E!3818 Project (2007–2009) with the financial support of Agence Nationale de Recherche (ANR) in France and the "Direction Générale des Technologies, de la Recherche et de l'Energie (DGTRE) de la Région Wallonne" in Belgium.

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