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Purification and Biochemical Characterization of an Acid-Stable Lipase from the Pyloric Caeca of Sardine (*Sardinella aurita*)

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Abstract A lipolytic activity was located in the sardine digestive glands (pyloric caeca), from which a sardine digestive lipase (SaDL) was purified. Pure SaDL has a molecular mass of 43 kDa as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis. The enzyme was found to be more active on short-chain triacylglycerols than on long-chain ones. SaDL does not present the interfacial activation phenomenon. Control experiments were performed under the same experimental conditions, with dromedary and turkey pancreatic lipases and showed a positive interfacial activation phenomenon. Sodium deoxycholate (NaDC) has an inhibitory effect on the lipase activity. The pure enzyme lost 40% of its activity in presence of 8 mM NaDC. SaDL was found to be mostly stable at low pH values. Interestingly, no colipase was detected in the sardine pyloric caeca. Analogous results were reported for the scorpion and the crab digestive systems. This is in line with the idea that colipase might has evolved in mammal animals simultaneously with the appearance of an exocrine pancreas. No similarity was found between the NH_2-terminal amino acid residues of SaDL and those of lipases from the digestive tract of other species. Altogether, these results suggest that SaDL is a member of a new group of lipases belonging to aquatic species.

Keywords *Sardinella aurita* · Pyloric caeca · Acid-stable lipase · Purification · Characterization

Abbreviations

SaDL	sardine digestive lipase
HPL	human pancreatic lipase
rDGL	recombinant dog gastric lipase

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DrPL	dromedary pancreatic lipase
TPL	turkey pancreatic lipase
SDL	scorpion digestive lipase
NaDC	sodium deoxycholate
AG	arabic gum
BSA	bovine serum albumin
PC	phosphatidylcholine

Introduction

Lipases (glycerol ester hydrolase EC 3.1.1.3) are found widely in the animal kingdom, as well as in plants and microorganisms such as bacteria and fungi [1–5]. These enzymes hydrolyze the carboxylic ester bonds of long-chain triacylglycerols which are present at lipid/water interfaces. They represent an important group of biotechnologically valuable enzymes.

Mammal lipases are well characterized [1, 2]. In contrast, much less is known about lipases from lower animals, such as fish. The presence of a lipase activity was detected in the pyloric caecum of cod [6]. The enzyme hydrolyzes triacylglycerols at an optimum pH value of 8.25 and an optimal temperature of 25 °C. Furthermore, another lipase was isolated from the intestines of a fish, *Cyprinion macrostomus* [7]. The molecular mass of the lipase was found to be 50 kDa. A specific activity of 254 U/mg was measured using an olive oil emulsion as substrate. The optimum pH value of the enzyme activity occurred between 6.5 and 9.0 [7].

The sardine is a pelagic fish which lives mainly in depths ranging between 10 and 80 m, along the coasts. The sardine digestive system (Fig. 1) begins by an esophagus. The heart is a muscle organ helping blood to circulate. The stomach, a dilated section of the digestive tract, and the intestine digest food and absorb nutrients [8]. The function of the pyloric caeca is not entirely understood, but it is known to secrete enzymes for food digestion and probably to absorb digested nutriments. The liver displays a number of physiological functions. It allows fat hydrolysis due to its endocrine enzymes and also serves as a storage organ for fats and carbohydrates [8]. The liver secretes also the bile that helps digestion. The spleen is an organ of the circulatory system where impurities present in the blood are destroyed.

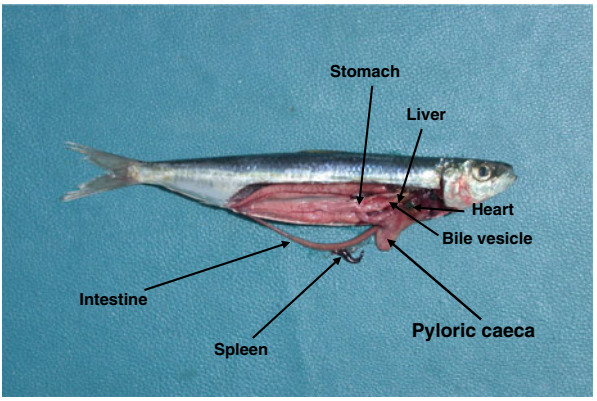

Fig. 1 Sardine used in this study (*Sardinella aurita*).*Ventral view* after dissection showing digestive apparatus including pyloric caeca used as starting material to purify the lipase

Many studies have attempted to purify proteases from the sardine intestines [9]. However, to our knowledge, no lipolytic enzymes have been purified from the sardine digestive glands so far. In this paper, we describe the purification and some biochemical characteristics of a fish lipase (sardine: *Sardinella aurita*) named sardine digestive lipase (SaDL) as part of ongoing research in our laboratory to discover new sources of these enzymes for potential food and biotechnological applications. In this study, the sardine pyloric caeca was used as starting material to detect the presence of a lipolytic activity.

Materials and Methods

Chemicals

Tributyrin (99%, puriss), benzamidine were from Fluka (Buchs, Switzerland); tripropionin (99%, GC) was from Janssen Chimica (Pantin, France), trioctanoin was from Acros Organics (Noisy-Le-Grand, France), triton X-100, β-mercaptoethanol, bovine serum albumin (BSA; 99%, puriss), and nitrocellulose membrane were from Sigma Chemical (St. Louis, MO, USA); arabic gum (AG) was from Mayaud Baker LTD (Dagenham, UK); sodium deoxycholate (NaDC) was from Sigma Chemical (St. Louis, MO, USA); tetrahydrolipstatin (THL) was a generous gift from Pr. A.F. Fischli (Buchs, Switzerland); acrylamide and bis-acrylamide electrophoresis grade were from Bio-Rad (Paris, France); marker proteins and supports of chromatography used for SaDL purification, Sephacryl S-200, Mono-Q Sepharose, and Mono-S Sepharose, were from Pharmacia (Uppsala, Sweden); protein sequencer Procise 492 cLC provided from Applied Biosystems (Roissy, France); pH-stat was from Metrohm (Herisau, Switzerland).

Sardines

Sardines (*S. aurita*) were bought from the fish market (Sfax, Tunisia). A stock of pyloric caeca removed immediately after dissection of the sardines was stored at −20 °C.

Enzymes

Turkey pancreatic lipase and scorpion digestive lipase are purified as described previously [10, 11].

Pure dromedary pancreatic colipase and lipase are generous gifts from Dr. Ben Bacha (Ecole Nationale d'Ingénieurs de Sfax (ENIS), Tunisie).

Recombinant dog gastric lipase (rDGL) was kindly provided by Meristem Therapeutics (Clermont-Ferrand, France).

Preparation of Pyloric Caeca Extract

Extraction buffer (20 mM sodium acetate, 150 mM NaCl, pH 5) was added to the pyloric caeca sample in the proportion of 10 ml per 1 g of fresh tissue. The mixture was then stirred with a magnetic bar during 30 min at 4 °C. The extract was centrifuged for 30 min at 12,000 rpm and the clear supernatant was collected to measure the lipase as well as the phospholipase activities.

Delipidation of Sardine Pyloric Caeca

After decongelation, pyloric caeca of sardine were delipidated according to the method described previously [12]. After delipidation, 9 g of powder were obtained from 68 g of fresh tissue.

Determination of Lipase Activity

The lipase activity was measured titrimetrically with a pH-stat, under the standard assay conditions described previously, using tributyrin as substrate: 0.25 ml tributyrin in 25 ml of 2 mM Tris-HCl, 50 mM NaCl, pH 9 [13]. Activity was also determined using as substrate olive oil emulsified with arabic gum under conditions previously reported [14]. Lipolytic activity was expressed as international units. Specific activities are expressed as unit per milligram of protein. One unit corresponds to 1 μmol of fatty acid released per minute.

Determination of Phospholipase Activity

The phospholipase activity was measured titratemetrically at pH 8 and 37 °C with a pH-stat, under the standard assay conditions described previously [15], using PC/bile salt liposomes as substrate in the presence of 3 mM NaDC, 7 mM $CaCl_2$, and 2 mM Tris-HCl. One unit of phospholipase activity was defined as 1 μmol of fatty acid released per minute.

Determination of Protein Concentration

Protein concentration was determined as described previously [16], using bovine serum albumin ($E_{1\ cm}^{1\%}$=6.7) as reference.

NH_2-terminal Sequence Analysis

The NH_2-terminal end of sardine digestive lipase was sequenced by automated Edman's degradation using an Applied Biosystems Protein sequencer Procise 492 cLC [17].

Effect of pH and Temperature on SaDL Activity and Stability

SaDL activity was tested in various buffers at different pH values [6–10] at 37 °C. The pH stability of the lipase was determined by incubating the enzyme at different pH values [3–9] for 30 min at room temperature. After incubation, the enzymatic solution was centrifuged to discard the insoluble material. The residual activity was determined under standard assay conditions.

The optimum temperature for the SaDL activity was determined by carrying out the enzyme assay at different temperatures (30–60 °C) at pH 9. The effect of temperature on lipase stability was determined by incubating the enzyme solution at different temperatures (25–50 °C) for 30 min. The residual activity was determined, after centrifugation, under standard assay conditions.

Lipases Antisera

Antisera directed each against one lipase (turkey pancreatic lipase, recombinant dog gastric lipase, or scorpion digestive lipase) were generous gifts from Dr. Zouari N. (LBGEL, Sfax, Tunisia).

SDS-PAGE and Immunoblotting Technique

Analytical polyacrylamide gel electrophoresis of proteins in the presence of sodium dodecyl sulfate (SDS/PAGE) was performed by the method of Laemmli [18]. The immunoreactivity of SaDL with lipases antisera (anti-TPL, anti-rDGL, or anti-SDL) was established by protein blotting. Proteins from SDS gel were transferred to nitrocellulose membranes. After the transfer, membranes were rinsed three times with phosphate buffer saline (PBS, 10 mM phosphate, 150 mM NaCl, pH 7.2), then saturated with 3% of milk powder in PBS (saturating buffer) for 1 h at room temperature. Thereafter, lipases antisera diluted at 1:1,000 with PBS containing 0.05% tween-20 (PBS/Tween-20) were incubated with the membranes for 1 h at room temperature. Afterwards, they were washed three times with PBS/tween-20 and incubated for 1 h at room temperature with a 1:2,000 dilution of alkaline phosphatase-conjugated anti-rabbit immunoglobulin (Sigma). After washing as mentioned above, membranes were incubated with substrate solution of phosphatase containing 0.3 mg/ml of nitroblue tetrazolium chloride (Sigma), 0.2 mg/ml of 5-bromo-4-chloro-3 indodylphosphate (Sigma), and 0.2 mg/ml of $MgCl_2$ to reveal the specific immunoreactivity.

Results and Discussion

Lipolytic Activity of the Sardine Pyloric Caeca

The lipase activity level measured in the sardine pyloric caeca extract was found to be 100 U/g of fresh digestive tissue using TC_4 as substrate. Furthermore, phospholipase activity was detected in the sardine pyloric caeca, using PC/bile salt liposomes as substrate and it was found to be around 20 U/g of fresh pyloric caeca. Therefore, sardine pyloric caeca are equipped with both lipase and phospholipase activities to achieve the hydrolysis of the major dietary lipids.

Purification of SaDL

Ninety sardines (Fig. 1) were used as a starting material for each purification procedure. Delipidated powder (9 g) of sardine pyloric caeca was suspended in 100 ml of buffer A: 20 mM sodium acetate pH 5, 20 mM NaCl, 2 mM benzamidine. The mixture was stirred during 45 min at 4 °C, then centrifuged for 30 min at 12,000 rpm.

Ammonium Sulfate Precipitation

The supernatant (85 ml) was brought to 65% saturation with solid ammonium sulfate under stirring conditions and maintained for 45 min at 4 °C. After centrifugation (30 min at 12,000 rpm), the pellet was resuspended in 10 ml of buffer A. Insoluble material was removed by centrifugation at 12,000 rpm during 15 min. Approximately 65% of the starting total activity of SaDL was recovered in the supernatant.

Filtration on Sephacryl S-200

The supernatant issued from ammonium sulfate precipitation was loaded on a gel filtration Sephacryl S-200 column (3×140 cm) equilibrated with buffer A. Elution of lipase was

performed with buffer A at 45 ml/h. The fractions containing the lipase activity eluted between 1.4 and 1.7 void volumes were pooled together (Fig. 2a).

Anion Exchange Chromatography

The pooled fractions containing SaDL activity issued from the Sephacryl S-200 column were concentrated and injected onto an FPLC Mono-Q column equilibrated with buffer B: 20 mM sodium acetate pH 5.8, 20 mM NaCl, 2 mM benzamidine. Non-fixed proteins were washed out with buffer B. The elution of the adsorbed proteins was then performed with a linear gradient of NaCl (50 to 350 mM of NaCl). SaDL activity was eluted at 150 mM NaCl (Fig. 2b). The recovery yield of SaDL activity after the Mono-Q column purification step was 20% of the starting enzymatic activity.

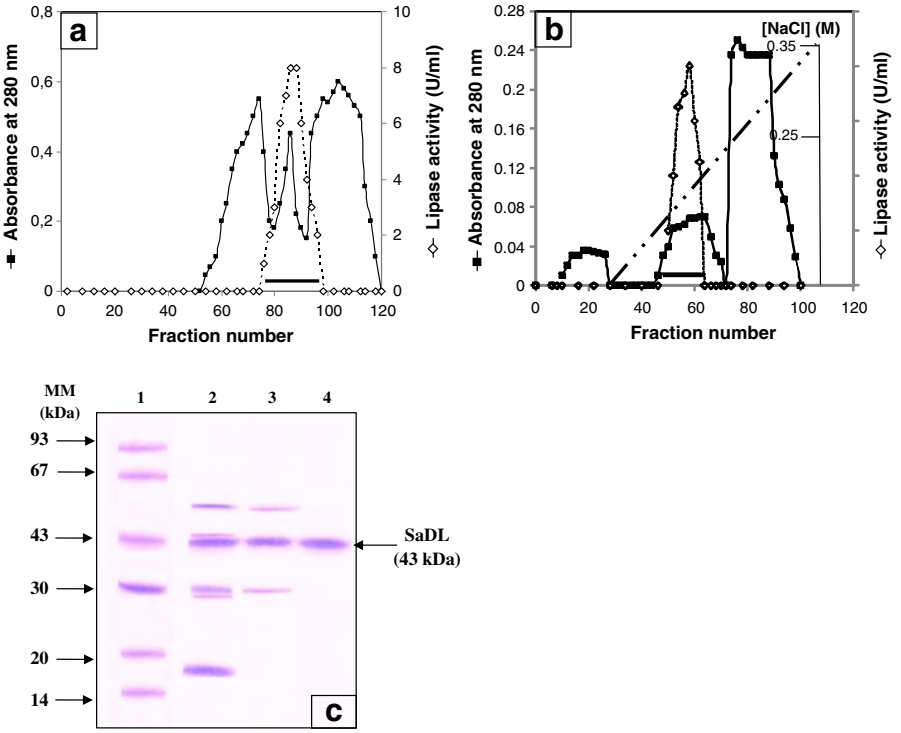

Fig. 2 **a** Chromatography of SaDL on Sephacryl S-200. The *column* (3×140 cm) was equilibrated with buffer A: 20 mM sodium acetate pH 5, 20 mM NaCl and 2 mM benzamidine. The elution of lipase was performed with the same buffer at a rate of 45 ml/h and fractions of 8 ml were collected. SaDL activity was measured as described in "Materials and Methods" section using TC_4 as substrate. Active fractions (76–102) were pooled. **b** Chromatography of SaDL on FPLC Mono-Q Sepharose. The column was equilibrated with buffer B: 20 mM sodium acetate pH 5.8, 20 mM NaCl, 2 mM benzamidine. The elution of the adsorbed proteins was then performed with a linear gradient of NaCl (50–350 mM). The flow rate was 120 ml/h and the fraction size was 3 ml. SaDL activity was measured as described in "Materials and Methods" section using TC_4 as substrate. Active fractions (54–70) were pooled. **c** Analysis of purified SaDL (SDS-PAGE (12%)). *Lane 1* molecular mass markers (Pharmacia); *lane 2* 10 μg of SaDL solution obtained after Sephacryl S-200 chromatography; *lane 3* 10 μg of SaDL solution obtained after Mono-Q Sepharose chromatography; *lane 4* 10 μg of purified SaDL obtained after the last step. The gel was stained with Coomassie blue

Filtration through a Cation Exchange Support

Active fractions eluted from the FPLC Mono-Q column were applied to a Mono-S column (2.5×6 cm) equilibrated with buffer A. Under these conditions, the enzyme was not adsorbed onto the cationic support and was recovered with the washing fractions, using the same buffer at 60 ml/h. The fractions containing the lipase activity were pooled.

This step resulted in fractions containing pure SaDL, as revealed by SDS/PAGE analysis (Fig. 2c). The purified enzyme was represented by a single band corresponding to a molecular mass of about 43 kDa. This result was in line with the molecular mass determined under native conditions, using gel filtration on FPLC column Sephacryl S-200 (data not shown). These data suggest that SaDL is a monomeric protein like all pancreatic lipases described in previous works [1].The purification flow sheet is shown in Table 1. The specific activity of the pure lipase reached 180 U/mg using TC_4 as substrate at pH 9 and 37 °C. Under the same conditions, a specific activity of 35 U/mg was obtained when using arabic gum emulsified olive oil as substrate. These results show that, in contrast to the fungal *Rhizopus oryzae* [19], the turkey pancreatic [10] and the dog gastric lipases [20], SaDL hydrolyzes more efficiently the short-chain than the long-chain triacylglycerols like all mammal [1] and chicken pancreatic lipases [21].

NH_2-terminal Sequence of SaDL

The NH_2-terminal sequencing of SaDL in solution allowed the unambiguous identification of 13 residues: LPGGAPMAAVPPL. It is noteworthy that the sequencing of the transferred SaDL band on a PVDF membrane gave the same aminoacids sequence. No similarity was found between the SaDL NH_2-terminal amino acid sequence and those of known lipases purified from the digestive tract of other species. SaDL appears to be a member of a new group of lipases belonging to the digestive glands of aquatic animals.

Effects of pH and Temperature on SaDL Activity and Stability

The effect of pH on enzyme activity was determined for pH values ranging from 6 to 10, using tributyrin as substrate. The purified enzyme was mostly active between pH 8 and 10, with an optimum at pH 9 (Fig. 3a). The same behavior was obtained with the turkey pancreatic lipase (TPL). Residual activity decreased significantly above pH 9.5 and only

Table 1 Flow sheet of SaDL purification.

Purification step	Total activity[a] (units)	Protein[b] (mg)	Specific activity (U/mg)	Activity recovery (%)	Purification factor
Extract of SaDL (9 g of delipidated powder)	3,000	1,579.0	1.9	100.0	1.0
$(NH_4)_2SO_4$ precipitation	2,650	1,104.0	2.4	88.3	1.3
S-200 chromatography	1,300	65.0	20.0	43.3	10.5
Mono-Q FPLC chromatography	600	8.82	68.0	20.0	35.8
Mono-S filtration	400	2.2	180.0	13.3	94.7

[a] One unit: 1 μmol of fatty acid released per minute using TC_4 as substrate.

[b] Proteins were estimated by Bradford method [16]. The experiments were conducted four times and standard errors are reported.

44% of the potential activity was measured at pH 10. Optimum pH values between 7 and 8.5 have been reported for lipase activities of fish species such as Indian major carp (*Labeo rohita*) [22] and cod (*Gadus morhua*) [6]. Unlike the TPL, the sardine lipase was found to be mostly stable in a broad range of acidic pH values (Fig. 3b). The lipase was stable at pH 5 after a 30-min incubation time, it maintains 90% of its maximal activity at pH 4 (Fig. 3b). Nevertheless, SaDL lost its activity upon incubation at pH values lower than 4 or higher than 8. Such large differences in pH optimum and pH stability were observed in the case of *Aspergillus* lipases. In the case of *Aspergillus niger*, maximum lipase activity was obtained at pH 2.5 and decreased significantly (50%) when the pH reached 4. However, the enzyme showed stability at alkaline pH range (pH 8–11) as it retains 100% of its original activity after incubation for 24 h [23]. Furthermore, a difference in pH optima and pH stability was reported also in the case of *Aspergillus*

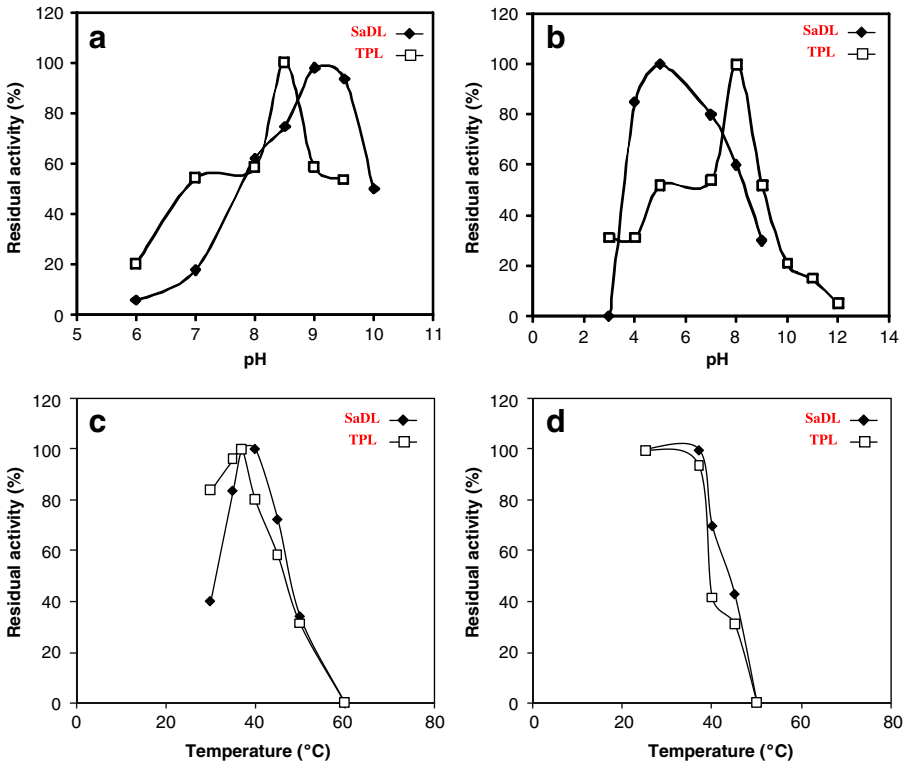

Fig. 3 For all measurements, the final enzyme concentrations were 31 nM for SaDL and 6 nM for TPL and the buffer used was 2 mM Tris-HCl, 50 mM NaCl. **a** Effect of pH on SaDL and TPL activities. Lipase activity was measured at 37 °C at various pH values [7–10] using TC_4 as substrate. **b** Effect of pH on SaDL and TPL stabilities. After 30 min incubation of the enzyme at various pH values using the following buffers: 0.5 M glycine-HCl (pH 3–4); 0.5 M sodium acetate (pH 5–6); 0.5 M Tris-HCl (pH 8–9), and 0.5 M sodium borate (pH 10–11), residual activity was measured at pH 9 and 37 °C using TC_4 as substrate. **c** Effect of temperature on SaDL and TPL activities. Lipase activity was measured at pH 9 at various temperatures using TC_4 as substrate. **d** Effect of temperature on SaDL and TPL stabilities. After 30 min incubation of the enzyme at various temperatures, residual activity was measured at pH 9 and 37 °C using TC_4 as substrate

nidulans where the lipase is most active at pH 6.5 and most stable at alkaline pH values (pH 7–9) [24].

The lipase activity was also determined at different temperatures under standard assay conditions (Fig. 3c). The SaDL, like the TPL, is active at temperatures varying from 35 °C to 45 °C with an optimum value at 37 °C. The optimum temperature for *S. aurita* lipase was similar to those described for the Indian major carp (*L. rohita*) [22] and the cod (*G. morhua*) [25] lipases and was higher than that of the rainbow trout lipase (20–25 °C) [26].

The thermal stability profile of the purified enzyme showed that the SaDL was thermosensitive since its activity was lost after 5 min incubation at a temperature higher than 55 °C (Fig. 3d), as it was found previously for other pancreatic lipases [1].

Interfacial Activation of SaDL

TC_3 was selected as a substrate to check whether SaDL displays the interfacial activation phenomenon, characteristic of the pancreatic lipases family [27]. The hydrolysis rate of TC_3 emulsified in 0.33% AG by SaDL as a function of substrate concentration shows a normal Michaelis–Menten dependence of the activity on the substrate concentration (Fig. 4). The maximal SaDL specific activity (100 U/mg) was reached at a TC_3 concentration that corresponds to the solubility limit. Under the same experimental conditions, it was observed that dromedary and turkey pancreatic lipases display low relative activity against soluble tripropionin (Fig. 4b). The lipase activity increases as the substrate concentration reaches the solubility limit. The maximal dromedary (DrPL) and turkey (TPL) pancreatic lipases specific activities (1,800 and 900 U/mg, respectively) were reached at a TC_3 concentration that exceeded the solubility limit. These results indicate that SaDL, unlike dromedary and turkey pancreatic lipases, does not exhibit the interfacial activation phenomenon.

However, as it was established in a previous study [27], true lipases were defined as a family of enzymes able to hydrolyze long-chain triacylglycerols independently of the presence or the absence of the interfacial activation phenomenon, originally described by Sarda and Desnuelle [28]. Hence, SaDL, which was found to hydrolyze olive oil, can be said to be a true lipase.

Effect of Amphiphiles on SaDL Activity

It is well known that amphiphiles (detergents, proteins) generally act as reversible "inhibitors" of pancreatic lipases [29]. Lipase inhibition was found to be correlated with a lack of lipase interfacial binding to its water insoluble substrates. The lack of lipase adsorption was due to the change of physicochemical properties of the lipid–water interface, following the binding of inhibitory amphiphiles [29]. Furthermore, bile salts act as physiological inhibitors of pancreatic lipases. This inhibition is reversed by a protein cofactor: the colipase [30]. Therefore, we checked the effect of increasing concentrations of bile salts (NaDC) on the rate of hydrolysis of TC_4 by SaDL. The SaDL activity decreased to reach 60% of its initial value at a NaDC concentration of 8 mM. The SaDL remains therefore active even at a bile salts concentration as high as 10 mM (data not shown). At a NaDC concentration of 8 mM, 60% of the initial activity was still observed. This type of behavior is in sharp contrast with all known mammalian pancreatic lipases which are strongly inhibited by bile salts, in the absence of colipase [1, 29]. Dromedary pancreatic colipase failed to reactivate the SaDL inhibited by bile salts. This suggests that sardine

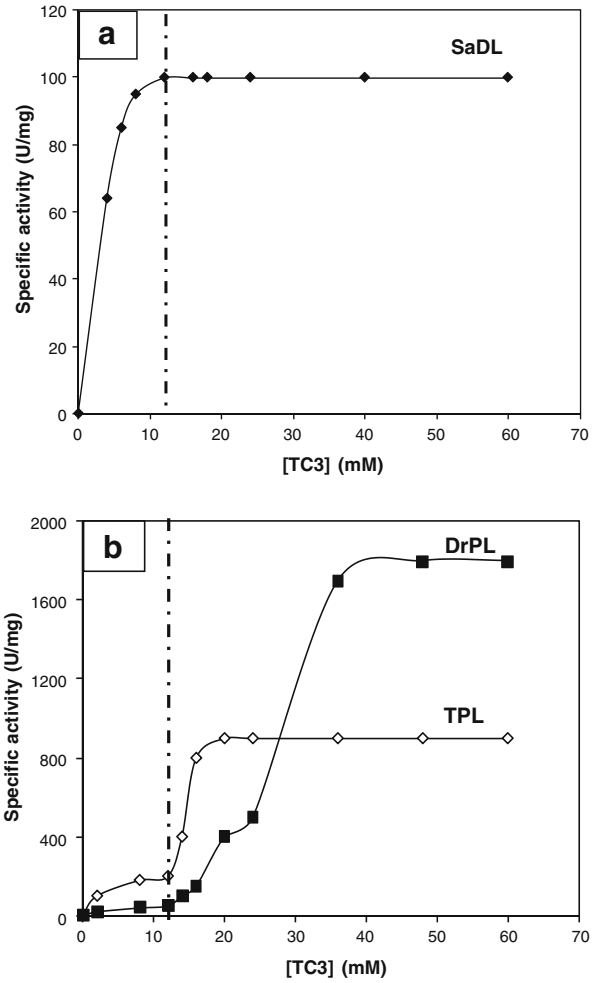

Fig. 4 **a** Specific activity of SaDL as function of TC_3 concentration. The TC_3 solutions were systematically prepared by mixing (3×30 s in a warring blender) a given amount of TC_3 in 30 ml of 0.33% AG and 0.15 M NaCl. **b** Specific activities of TPL and DrPL as function of TC_3 concentration. The TC_3 solutions were systematically prepared by mixing (3×30 s in a warring blender) a given amount of TC_3 in 30 ml of 0.33% AG and 0.15 M NaCl. The release of propionic acid was recorded continuously at pH 7 and 37 °C using a pH-stat. The limit of TC_3 solubility (12 mM) is indicated by *vertical dotted lines*

digestive system does not contain the classical lipase/colipase complex known in mammal animals. Analogous results were reported for the scorpion and the crab digestive systems [11, 31]. Furthermore, our results seem to differ from previous findings claiming that bile salts are required for lipase activities of many fish species. In fact, Gjellesvik et al. [6] had reported that a partly purified lipase from cod (*G. morhua*) showed an absolute dependency for bile salts (crude from ox bile) when olive oil and tributyrin were used as substrates at pH 8.5. Furthermore, a lipase from leopard shark, characterized as a crude pancreatic preparation, demonstrated an absolute requirement for bile salts for its activity with natural bile salts of the shark when trioleoylglycerol was used as substrate at 36 °C and pH 8.5 [32].

Furthermore, the effect of varying concentrations of amphiphilic proteins like BSA on the rate of hydrolysis of TC_4 by SaDL was studied. SaDL activity decreased rapidly with increasing the BSA concentration (data not shown). This behavior recalls that of pancreatic lipases which were fully inhibited by BSA when TC_4 is used as substrate [33, 34]. The addition of increasing concentrations of NaDC to the TC_4 lipolysis medium containing SaDL and inhibitory concentration of BSA (60 μM) fully restored the lipolytic activity (data not shown). This reversible inhibitory effect of SaDL activity by amphiphilic proteins recalls similar findings obtained with pancreatic lipases by Gargouri et al. [34]. This inhibition by amphiphiles is likely to be a general phenomenon related to desorption of the lipase from its substrate occurring after a change in the interfacial quality due to the amphiphilic protein, as it was suggested for pancreatic lipases.

SaDL is a Serine Enzyme

It was reported that THL acts in vitro as a potent inhibitor of pancreatic and gastric lipases [35], reacting with their catalytic serine. In order to check if the SaDL is a serine enzyme, we studied the effect of THL on the lipase activity (Fig. 5). The SaDL was incubated at pH 5.0 and 25 °C in the presence of THL (molar excess of 100) and the residual lipase activity was measured. Figure 5 shows that SaDL lost more than 70% of its initial activity when incubated during 15 min with THL.

It is worth noting that SaDL was not completely inactivated by a large molar excess of THL. Adding bile salts to the incubation medium accelerated the inactivation rate of SaDL by THL due to the fact that THL probably formed mixed bile salt/THL micelles. As in the case of lipase substrates, the presence of mixed bile salts/THL micelles may give rise to a better "interfacial quality," thus improving the lipase adsorption and/or increasing the THL

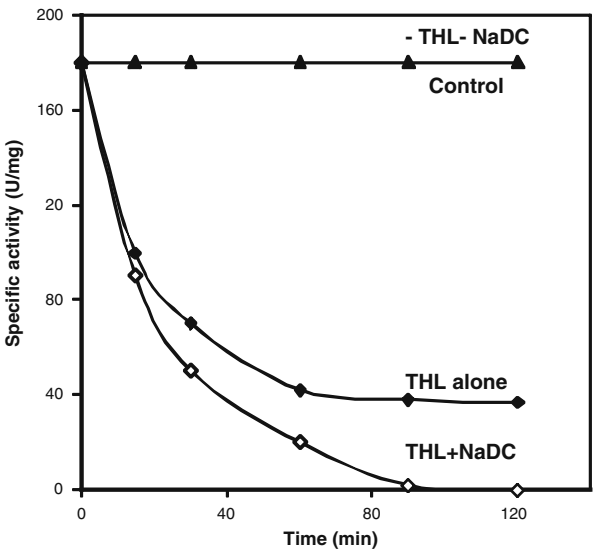

Fig. 5 Inhibition of SaDL by THL in the absence or presence of 4 mM NaDC. SaDL was incubated at 25 °C with THL (molar ratio THL/SaDL=100). SaDL activity was measured at pH 9 and 37 °C using TC_4 as substrate. The control experiment in the absence of THL and NaDC (−THL–NaDC) was showed. The experiments were conducted four times

accessibility, as it was previously suggested for other lipases [36, 37]. In the absence of THL and bile salts, the activity of SaDL was not affected.

It can thus be suggested that SaDL is a serine enzyme, like all known lipases purified from various sources.

Immunochemical Properties

Immunological cross-reactivity of SaDL with anti-turkey pancreatic lipase (anti-TPL), anti-scorpion digestive lipase (anti-SDL), and anti-recombinant dog gastric lipase (anti-rDGL) sera were carried out using the Western blotting technique. Native SaDL was strongly recognized by anti-rDGL serum and no cross-immunoreactivity was detected with anti-TPL or anti-SDL sera (Fig. 6). These results might be explained by the fact that SaDL shares common antigenic determinants with gastric lipases.

Conclusion

The purification of a lipase from a fish, the sardine, is described for the first time in this paper. The pure enzyme, tentatively named sardine digestive lipase (SaDL), is a monomer with a molecular mass of 43 kDa. It was found to hydrolyze more efficiently short-chain triacylglycerols than long-chain ones. Unlike the pancreatic lipases, SaDL do not display the interfacial activation phenomenon; it expresses its full activity in the absence of any cofactor. The sardine lipase is more stable at acidic pH values than pancreatic lipases. The NH_2-terminal sequence of the SaDL shows no similarities with known lipases. The SaDL appears to be a member of a new group of lipases belonging to the digestive tract of aquatic species.

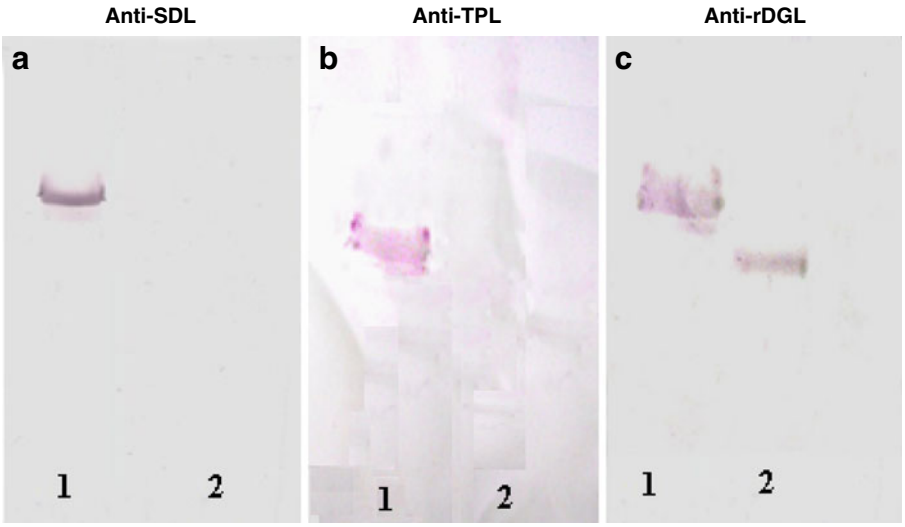

Fig. 6 Immunoblot analysis of SaDL with scorpion digestive lipase (SDL), turkey pancreatic lipase (TPL) and recombinant dog gastric lipase (rDGL). **a** Immunoreactivity of SDL [1], 15 µg and SaDL [2], 15 µg with a serum *anti-SDL*. **b** Immunoreactivity of TPL [1], 15 µg and SaDL [2], 15 µg with a serum *anti-TPL*. **c** Immunoreactivity rDGL [1], 15 µg and SaDL [2], 15 µg with a serum *anti-rDGL*

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