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Biochimica et Biophysica Acta 1726 (2005) 67 – 74
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Purification and characterization of a novel lipase from the digestive glands of a primitive animal: The scorpion

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Received 31 March 2005; received in revised form 29 July 2005; accepted 1 August 2005
Available online 31 August 2005

Abstract

Higher animal's lipases are well characterized, however, much less is known about lipases from primitive ones. We choose the scorpion, one of the most ancient invertebrates, as a model of a primitive animal. A lipolytic activity was located in the scorpion digestive glands, from which a scorpion digestive lipase (SDL) was purified. Pure SDL, a glycosylated protein, has a molecular mass of 50 kDa, it presents the interfacial activation phenomenon. It was found to be more active on short-chain triacylglycerols than on long-chain triacylglycerols. SDL is a serine enzyme and possesses one accessible sulfhydryl group which is not essential for the catalysis. Among the NH_2-terminal 33 residues, a 17 amino acids sequence shows similarities with sequence of *Drosophila melanogaster* putative lipase. Interestingly, neither colipase, nor bile salts were detected in the scorpion hepatopancreas. This indicates that colipase evolved in vertebrates simultaneously with the appearance of an exocrine pancreas and a true liver which produces bile salts. Furthermore, polyclonal antibodies directed against SDL failed to recognise the classical digestive lipases. Altogether, these results suggest that SDL is a member of a new group of digestive lipases belonging to invertebrates.

Keywords: *Scorpio maurus*; Scorpion; Hepatopancreas; Digestive lipase; Purification; Characterization

1. Introduction

Lipases are hydrolytic enzymes (EC 3.1.1.3) that break down triacylglycerols into free fatty acids and glycerol and operate at the surface of emulsified lipid substrates.

Higher animal's lipases are well characterized [1–4], in contrast much less is known about lipases from lower ones, such as crustaceans: The presence of lipase activity was detected in the gastric juice of *Homarus americanus*. The enzyme was found to hydrolyse tributyrin (TC_4) twice as fast as triolein [5]. Furthermore, another lipase was isolated from an insect, *Cephaloleia presignis* [6]. The purified enzyme, which has a molecular mass of 31 kDa, showed no similarity with any known lipases. Recently, a lipase, showing a strong antiviral activity, was purified from the digestive juice of silkworm, *Bombyx mori* [7]. Moreover, different putative lipases from several insects like *Drosophila melanogaster* [8] and *Anophaeles gambiae* [9] have been cloned and sequenced. These lipases show sequence similarities with mammalian lipases.

Within the phylum of Arthropoda, scorpions are ancient chelicerates that have changed little since the Silurian (450 million years) [10], and are considered as the oldest known terrestrial species. Many studies have described the morphology as well as the digestive system of scorpions [11–17]. Scorpions are nocturnal predators feeding primarily on insects and other arthropods. Digestion begins externally and is completed in the digestive glands. In fact, food is captured by the pedipalps, torn apart and held in the preoral cavity by the chelicerae, and masticated by the gnathobases. Hydrolytic enzymes flood onto food from glands in the gnathobases. Hydrolysis begins before

Abbreviations: AG, Arabic gum; CM-Sephadex, carboxymethyl Sephadex; DEAE, diethylaminoethyl; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ELISA, enzyme linked immunosorbent assay; FPLC, fast pressure liquid chromatography; NaDC, sodium deoxycholate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TC_3, tripropionin; TC_4, tributyrin; THL, tetrahydrolipstatin; pAbs, polyclonal antibodies; SDL, scorpion digestive lipase; OPL, ostrich pancreatic lipase; rDGL, recombinant dog gastric lipase; TPL, turkey pancreatic lipase

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doi:10.1016/j.bbagen.2005.08.005

ingestion, and the resulting pre-digested mixture is sucked into the gut. The gut runs the length of the body from the mouth at the anterior end of the cephalotorax (prosoma) to the anus at the posterior end of the metasoma. In the preabdomen (mesosoma), the midgut is a simple endodermal tube from which arise many pairs of diverticuli known as digestive glands or hepatopancreas. These glands occupy most of the space in the mesosoma and are conspicuous, clumped together and cannot usually be distinguished as separate glands. The essential of digestion occurs in these digestive glands.

This mesosoma cleared of the cuticle represents the starting material in this study to check the existence of a lipolytic activity. Few studies have attempted to purify proteins like antimicrobial peptides and protease inhibitor [18,19]. However, to our knowledge, no lipolytic enzymes from the scorpion digestive glands have been described so far.

This paper will report the purification to homogeneity of an active lipase from the scorpion hepatopancreas. This lipase, tentatively named: scorpion digestive lipase (SDL) was characterized with respect to its biochemical properties.

2. Materials and methods

2.1. Animals

Scorpions (chelicerate, scorpionidae, *Scorpio maurus*) were collected alive from the area of Agareb (Sfax, Tunisia) and frozen until death.

2.2. Enzymes

Pure ostrich (OPL) and Turkey (TPL) pancreatic lipases and ostrich colipase were 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 and Jouveinal/Parke Davis, France.

2.3. Delipidation of scorpion digestive glands

After decongelation, mesosomas which contained the scorpion digestive glands, were cleared from the cuticle (Fig. 1) and delipidated according to the method described previously [20]. After delipidation, 15 g of powder were obtained from 60 g of fresh tissue.

2.4. Lipase activity determination

The lipase activity was measured titrimetrically at pH 9 and 37 °C with pH-Stat (Metrohm, Switzerland) using TC_4 (0.25 ml of TC_4 in 25 ml 2 mM Tris–HCl, 150 mM NaCl and 1 mM NaDC) or olive oil emulsion (1 ml of olive oil emulsified in 25 ml, 3% AG, 150 mM NaCl by mixing mechanically twice for 1 min using the Warning Blendor system) as substrate [21]. Some lipase assays were performed in the presence or in the absence of NaDC or colipase. Lipase activity was also measured using tripropionin (TC_3) as substrate [22]. One lipase unit corresponds to 1 μmol of fatty acid released per minute.

2.5. Determination of protein concentration

Protein concentration was determined as described in previous work [23] using bovine serum albumin ($E^{1\%}_{1\,cm}$=6.7) as reference.

2.6. Amino acid sequencing

The NH_2-terminal end of SDL was sequenced by automated Edman's degradation, using an Applied Biosystems Protein Sequencer Procise 492 cLC

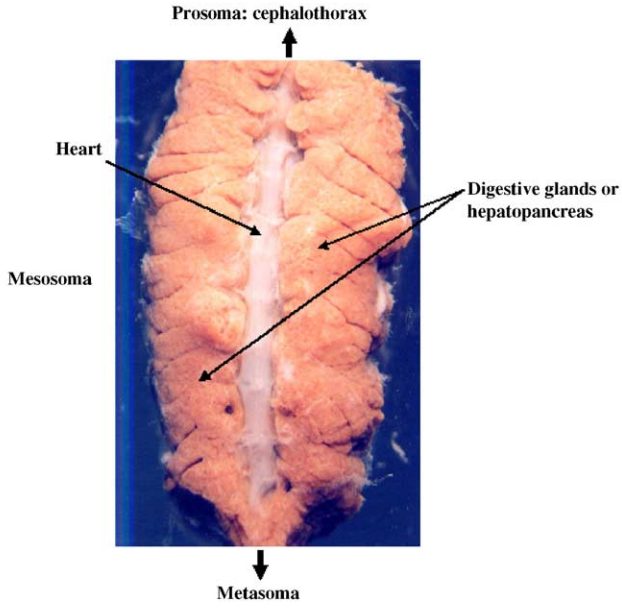

Fig. 1. Dorsal view of *Scorpio maurus* hemocoel of the mesosoma cleared from the cuticle enlarged 10 times.

[24]. The sequence was kindly determined by Dr. Reinbolt (Institut de Biologie Moleculaire et Cellulaire (IBMC), UPR 9002 Centre National de Recherche Scientifique (CNRS), Strasbourg, France) and confirmed using samples issued from three different purifications by Dr. Mejdoub (Faculté des Sciences de Sfax (FSS), Tunisie).

2.7. Oligosaccharides content

The glycan chains content in the purified SDL was estimated by anthrone-sulfuric acid method using glucose as a standard [25].

2.8. Bile salts titration

The presence of bile salts concentration was checked as described in previous work [26].

2.9. Reaction of DTNB with SDL

A 0.7-ml cuvette was filled with 0.6 ml of 0.1 M Tris–HCl buffer (pH 8) containing 0.7 mg of pure SDL and a 20-molar excess of DTNB. The number of titrated groups was calculated from the maximal absorbance using molar extinction coefficient values of 13600 at 412 nm for TNB [27]. At the same time, residual SDL activity was measured in aliquots taken from the reaction mixture. In the absence of sulfhydryl reagent, the lipase activity remained stable during the incubation period (data not shown).

2.10. Production of polyclonal antibodies and ELISA analysis

Polyclonal antibodies (pAbs) directed against SDL were produced using rabbits. Rabbits were injected subcutaneously and intra-muscularly every 3 weeks with 0.5 mg of the purified lipase. The first injection included complete Freund's adjuvant, while the last two injections contained incomplete adjuvant. The immunoreactivity of anti-SDL pAbs with rDGL and OPL was checked using the ELISA technique [28].

2.11. SDS-PAGE and Western blotting

Analytical polyacrylamide gel electrophoresis of proteins in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed by the method of Laemmli