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Substrate specificity and kinetic properties of enzymes belonging to the hormone-sensitive lipase family: Comparison with non-lipolytic and lipolytic carboxylesterases

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Abstract

We have studied the kinetics of hydrolysis of triacylglycerols, vinyl esters and *p*-nitrophenyl butyrate by four carboxylesterases of the HSL family, namely recombinant human hormone-sensitive lipase (HSL), EST2 from *Alicyclobacillus acidocaldarius*, AFEST from *Archeoglobus fulgidus*, and protein RV1399C from Mycobacterium tuberculosis. The kinetic properties of enzymes of the HSL family have been compared to those of a series of lipolytic and non-lipolytic carboxylesterases including human pancreatic lipase, guinea pig pancreatic lipase related protein 2, lipases from *Mucor miehei* and *Thermomyces lanuginosus*, cutinase from *Fusarium solani*, LipA from *Bacillus subtilis*, porcine liver esterase and Esterase A from *Aspergilus niger*. Results indicate that human HSL, together with other lipolytic carboxylesterases, are active on short chain esters and hydrolyze water insoluble trioctanoin, vinyl laurate and olive oil, whereas the action of EST2, AFEST, protein RV1399C and non-lipolytic carboxylesterases is restricted to solutions of short chain substrates. Lipolytic and non-lipolytic carboxylesterases can be differentiated by their respective value of $K_{0.5}$ (apparent K_m) for the hydrolysis of short chain esters. Among lipolytic enzymes, those possessing a lid domain display higher activity on tributyrin, trioctanoin and olive oil suggesting, then, that the lid structure contributes to enzyme binding to triacylglycerols. Progress reaction curves of the hydrolysis of *p*-nitrophenyl butyrate by lipolytic carboxylesterases with lid domain show a latency phase which is not observed with human HSL, non-lipolytic carboxylesterases, and lipolytic enzymes devoid of a lid structure as cutinase.

Keywords: Carboxylesterase; EST2; AFEST; Hormone-sensitive lipase; HSL family; Kinetic study

1. Introduction

Carboxylesterases (carboxylesterases) include two classes of enzymes widely represented in all living organisms, commonly designated as esterases and lipases. Esterases and lipases were first differentiated on the basis of their substrate specificity. It was early established that esterase activity was restricted to aqueous solutions of short acyl chain esters whereas lipases specifically hydrolyzed emulsions of water insoluble triacylglycerols [1]. Later, an alternative classification of carboxylesterases based on their amino acid sequence homology has been proposed. According to Hemila et al. [2], enzymes of the esterase/lipase superfamily have been divided into three families, namely the LPL family, which includes lipoprotein lipase, hepatic and pancreatic lipases, the EST family (cholinesterase and lipases from *Geotrichum candidum* and *Candida rugosa*) and the hormone-sensitive lipase (HSL) family. The HSL family includes mammalian HSL and several proteins of bacterial origin among which are brefeldin A esterase (BFAE) from *Bacillus subtilis* [3], heroin esterase from *Rhodococcus* sp. strain H1 [4], acetyl hydrolase from *Streptomyces* viridochromogenes [5], lipase 2 from *Moraxella* TA 114 [6,7] and two thermophilic carboxylesterases, namely

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EST2 from *Alicyclobacillus acidocaldarius* [8] and AFEST from *Archeoglobus fulgidus* [9]. More recently, several other proteins of the HSL family, as the protein Rv1399c protein from *Mycobacterium tuberculosis* (protein Rv1399c) have been biochemically characterized [10].

Enzymes of the HSL family contain the consensus sequence Gly-X-Ser-X-Gly containing the active residue of serine which, together with an histidine and an aspartic, or glutamic acid, form the charge relay network of the catalytic triad common to all carboxylesterases and serine proteases. Apart from this sequence, they contain the His–Gly–Gly–Gly sequence pattern located at about 70–100 amino acids upstream of the catalytic serine. This sequence contributes to the formation of the so-called oxyanion hole.

Members of the HSL family have an average molecular mass of 34–40 kDa whereas that of HSL is in the range of 85–120 kDa. A two-domain structure has been proposed for HSL [11]. The N-terminal domain, which contains around 300 amino acids, shows no sequence homology to any other protein. It is generally assumed that this domain mediates the interaction of HSL with adipocyte lipid-binding protein [12]. The C-terminal domain of HSL (around 450 residues), known as the catalytic domain, contains the residues of the catalytic triad [13,14]. It shows significant identity with carboxylesterases of the HSL family of lower molecular mass. In HSL, this region contains a large sequence stretch of about 150 amino acids known as the regulatory module where are located the phosphorylation sites of the enzyme. Recent studies have suggested that specific sequences within the C-terminal region of the regulatory module of HSL is involved in a lipid pocket and is crucial for determining lipolytic activity [15].

The three-dimensional (3D) structure of several bacterial enzymes of the HSL family including BFAE [3], EST2 [16] and AFEST [9], have been determined in the recent years. Crystallographic investigations have identified a common protein fold shared with other hydrolytic enzymes, known as the α/β hydrolase fold. This particular structure is characterized by a central parallel β-sheet core domain, surrounded on both sides by α-helices, which contain the residues of the catalytic triad. In addition, the 3D-structure of BFAE, EST2 and AFEST shows the presence of two separate helical regions, external to the α/β core, that cover the active site in such a way that free access of a long chain triacylglycerol molecule is impaired. This structure is reminiscent of the lid domain found in well-characterized long chain triacylglycerol hydrolyzing enzymes as fungal lipases from *Thermomyces lanuginosus* (TLL) or *Mucor miehei* (MML), and mammalian lipases of the LPL family [17–19]. As yet, no crystal structure of HSL has been reported and no putative lid domain has been localized in the protein. However, recent studies on the inhibition of HSL by specific serine enzyme inhibitors, as diethyl-*p*-nitrophenyl phosphate (E600), or phenylmethylsulfonyl fluoride (PMSF), however, have shown that HSL, in sharp contrast to classical lipases possessing a lid preventing free access to active serine, reacts with these inhibitors in the absence of micelles of detergent, which suggests that HSL lacks this domain [20]. On the other hand, superimposition of the crystallograhic structures of a mutant of EST2 and of the lipase from *Candida rugosa* (CRL), in its closed conformation, shows the concomitant occurrence of the CRL lid and the N-terminal region of EST2. In both proteins, these structures cover the active site but in opposite relative orientation and it is generally agreed that the two structures have different functions [21]. For EST2 from *A. Acidocaldarius*, it was recently reported that a deletion of 35 amino acids at the N-terminus has a dramatic effect on the kinetic properties of the enzymes [22].

To date, only few comparative kinetic studies of the carboxylesterases of the HSL family have been reported. In this study, we report the kinetic properties of four enzymes of the HSL family, namely recombinant human HSL, EST2, AFEST and protein Rv1399c. Results obtained with triacylglycerols and vinyl esters substrates have been compared to those collected with various lipolytic and non-lipolytic carboxylesterases including human pancreatic lipase (HPL) and guinea pig pancreatic lipase related protein 2 (GPL-RP2), lipases from *Mucor miehei* and *Thermomyces lanuginosus*, cutinase from *Fusarium solani*, LipA from *Bacillus subtilis*, porcine liver esterase and Esterase A from *Aspergilus niger*, using triacylglycerols, vinyl esters and p-nitrophenyl butyrate (*p*-NPB) as substrates.

Our results show that EST2 and AFEST display substrate specificity and kinetic properties similar to those of non-lipolytic carboxylesterases, in contrast to human HSL whose kinetic behaviour is similar to that of lipolytic enzymes. They further confirm that non-lipolytic and lipolytic carboxylesterases can be differentiated by their apparent K_m ($K_{0.5}$) for the hydrolysis of short acyl chain ester substrates [23].

2. Materials and methods

2.1. Chemicals

Triacylglycerols (triacetin, tripropionin, tributyrin and trioctanoin) were supplied by Acros-Organics (F-9316 Noisy-le-Grand, France). Olive oil was from local origin. Vinyl esters (vinyl acetate, vinyl propionate, vinyl butyrate and vinyl laurate) and *p*-NPB were purchased from Sigma-Aldrich-Fluka (F-38297 St-Quentin-Fallavier, France). The solubility limit of triacetin, tripropionin and tributyrin, in 2.5 mM Tris–HCl buffer, pH 7.5, with 0.1 M NaCl, is 330 mM, 10 mM and 0.4 mM, respectively, and that of vinyl acetate, vinyl propionate and vinyl butyrate is 315 mM, 86 mM and 22 mM, respectively [23]. The solubility limit of *p*-NPB is 1 mM [24].

2.2. Enzymes

Recombinant human HSL was expressed and purified from baculovirus infected insect cells as previously described [20]. Protein Rv1399c [10] was prepared by Dr. S. Canaan at the laboratory. Pure EST2 and AFEST were produced at the Institute of Protein Biochemistry, Napoli, Italy [8,9]. Protein Esterase A (EstA) from *Aspergillus niger* [25] was a gift of Dr. Y. Bourne (CNRS, Marseille, France). Porcine liver esterase (PLEst) was from Sigma-Aldrich-Fluka. HPL, GPL-RP2 and porcine colipase were provided by Dr F. Carriere (CNRS, Marseille, France). Recombinant *Fusarium solani* cutinase was a gift of Dr. M. Egmond (Utrecht University, The Netherlands). MML and TLL were provided by Dr. S. Patkar (Novo Nordisk A/S, Bagsvaerd, Denmark). *Bacillus subtilis* LipA was a gift from Dr. W. Quax (Groningen, The Netherlands).