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Research paper

The SGNH-hydrolase of *Streptomyces coelicolor* has (aryl)esterase and a true lipase activity

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Abstract

The *Streptomyces coelicolor* A3(2) gene SCI11.14c was overexpressed and purified as a His-tagged protein from heterologous host, *Streptomyces lividans*. The purification procedure resulted in 34.1-fold increase in specific activity with an overall yield of 21.4%. Biochemical and physical properties of the purified enzyme were investigated and it was shown that it possesses (aryl)esterase and a true lipase activity. The enzyme was able to hydrolyze *p*-nitrophenyl-, α- and β-naphthyl esters and poly(oxyethylene) sorbitan monoesters (Tween 20–80). It showed pronounced activity towards *p*-nitrophenyl and α- and β-naphthyl esters of C_{12}–C_{16}. Higher activity was observed with α-naphthyl esters. The enzyme hydrolyzed triolein (specific activity: 91.9 U/mg) and a wide range of oils with a preference for those having higher content of linoleic or oleic acid (C18:2; C18:1, *cis*). The active-site serine specific inhibitor 3,4-dichloroisocoumarin (DCI) strongly inhibited the enzyme, while tetrahydrofurane and 1,4-dioxane significantly increased (2- and 4- fold, respectively) hydrolytic activity of lipase towards *p*-nitrophenyl caprylate. The enzyme exhibited relatively high temperature optimum (55 °C) and thermal stability. CD analysis revealed predominance of α-helical structure (54% α-helix, 21% β-sheet) and a T_m value at 66 °C.

Systematic bioinformatic analysis of deduced amino acid sequence of *S. coelicolor* enzyme placed it to the SGNH-hydrolase family. Phylogenetic analysis of the predicted protein homologous to the *S. coelicolor* SGNH-hydrolase generated three distinct groups consisting of proteins from Actinomycetales, Ascomycota and Nematoda. At present it seems that these enzymes are most conserved among soil inhabiting organisms.

Keywords: *Streptomyces coelicolor*; GDSL/SGNH family; Lipase; (Aryl)esterase

1. Introduction

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are ubiquitous enzymes that have a significant physiological role in the metabolism of fats and oils and wide-ranging biotechnological application, and as such they remain a subject of intensive study [1]. Bacterial lipolytic enzymes have been classified into eight families according to their amino acid sequence and biological properties [2]. The enzymes of group II do not possess a conventional motif G-X-S-X-G around the active-site serine located near the centre of the sequence, rather they have an active-site serine in the conserved GDSL block close to the N-terminus [3]. This group of serine esterases/lipases was denoted as the GDSL family. Recently, detailed sequence and structural analysis of the GDSL family led to the designation of some GDSL enzymes as SGNH

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hydrolases. This enzyme family was defined by four invariant residues, serine (**S**), glycine (**G**), asparagine (**N**), and histidine (**H**) in blocks I, II, III and V, respectively [4,5]. The GDSL family comprises a diverse group of hydrolases including lipases, esterases, thioesterases, arylesterases, proteases and lysophospholipases. These enzymes display various functional properties such as broad substrate specificity and regiospecificity [5]. In comparison to the common lipases, the GDSL lipases have a flexible active site that changes conformation in the presence of substrate [5], similarly to the induced-fit mechanism proposed by Koshland [6]. Although knowledge on this class of enzymes is accumulating, elucidation of the biological functions/roles of GDSL hydrolases and better understanding of their reaction mechanisms, including 3-D structure information are still needed.

Streptomycetes are filamentous saprophytic Gram-positive bacteria with high G + C content, ubiquitous in soil and best known as producers of antibiotics. During the last few decades several reports described screening for lipolytic activities among different members of this genus [7,8]. The available genome data bases for streptomycetes predicted a large number of genes encoding enzymes of different lipolytic activities (approximately 50–80). Although these data point out the potential for synthesis of a broad spectrum of lipases, only a few have been studied and reported so far. Three homologous lipases were found in *Streptomyces exfoliatus* M11 [9], *Streptomyces albus* G [10] and *Streptomyces coelicolor* A3(2) [11]. In addition, one non related lipase was found in *Streptomyces cinnamomeus* [12] and another in *Streptomyces rimosus* [13]. The latter represents the best biochemically characterized GDSL/SGNH lipase from streptomycetes and it was the main object of our studies over several years [13–18]. DNA analysis demonstrated the existence of similar gene(s) in distantly related *Streptomyces* species [16], which was in concert with genome mining: two similar GDSL lipase genes were found in *S. coelicolor* A3(2) [19], one in *Streptomyces avermitilis* [20] and three in the recently reported genome of *Streptomyces griseus* [21]. Most recently Côté and Shareck [22] have reported cloning and basic biochemical characterization of two GDSL lipases (SCO1725 and SCO7513) from *S. coelicolor* A3(2).

In this study we investigated in detail the properties of GDSL/SGNH lipase (SCO1725) from *S. coelicolor* A3(2). We report here the facile purification of His-tagged *S. coelicolor* lipase, phylogenetic studies and extensive biochemical characterization. Our results are compared with data on *S. rimosus* lipase, and the impact of the His-tag on enzyme properties is discussed.

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

Escherichia coli XL1-Blue (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI*^{q} ZdeltaM15 Tn*10* (Tet^r)]) and GM119 (*F− fhuA2 lacY1 galK2 glnV44 galT22 metB1 mtlA2 dam-3 dcm-6 tsx-78 λ-*) was used for cloning and as a source of non methylated DNA. *S. coelicolor* A3(2) that possesses gene encoding lipase (SCO1725) throughout this manuscript was shortened to *S. coelicolor*. *Streptomyces lividans* TK23 (Spc^R) was used for lipase production.

Plasmids used in this study: pET-15b (Novagen, Germany) and bifunctional *E. coli*/*Streptomyces* vector pANT849pWB19N [23].

E. coli was grown at 37 °C in LB medium [24]. Streptomycetes were grown at 30 °C in liquid media: CRM (103 g sucrose, 10.12 g $MgCl_2 \times 6H_2O$, 15 g tryptic soy broth (TSB), 5 g yeast extract and 300 μL 2.5 N NaOH per 1 L, pH ~ 7.2) for plasmid isolation or inoculum preparation, and GR_2d (7 g $CaCO_3$, 40 g dextrin, 8 g CSS, 2 g $(NH_4)_2SO_4$, 280 μL lactic acid, 10 mL 1 M Tris–HCl, per 1 L of tap water, pH 8.0 was adjusted with 6 N NaOH; autoclaved twice) for lipase production. Solid media were: MS for sporulation and R5 for protoplast regeneration [25].

When appropriate, ampicillin, thiostreptone or kanamycin (Sigma, USA) were added to the media at a final concentration of 100, 25, or 10 μg/mL, respectively. Only to MS medium thiostreptone was added at concentration of 50 μg/mL.

2.2. DNA manipulation, cloning and expression

Standard molecular biology protocols [24] were applied to clone SCI11.14c gene (here abbreviated as *scL*) encoding lipase (SCO1725). QIAprep Spin Miniprep Kit (QIAGEN, Germany) was used to purify plasmids from *E. coli*, and alkaline lysis procedure [25] from *S. lividans*. GFX PCR DNA/Gel Band Purification Kit (GE Healthcare, USA) was used to purify PCR products. Restriction enzymes and T4 DNA ligase (Fermentas, USA) were used according to manufacturer's instructions. Sequencing was performed with automatic sequence analyzer "ABI PRISM® 3100-Avant Genetic Analyser" (Applied Biosystem, USA) using "ABI PRISM BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit".

Gene SCI11.14c (*scL*) was amplified by PCR using *S. coelicolor* A3(2) cosmid I11 [26] as template and following primers: forward (5′-CGGAATTC**GGAGG**TTCC**ATG**AGACGTTTCCGACTTGTCGGCTTCCTGA-3′; EcoRI underlined, ribosomal binding site (RBS) and start codon bolded) and reverse (5′-CCCCAAGCTT**TCA**$(GTG)_6$GGCGGCGCCGTTGAGGACGG-3′; HindIII underlined, stop codon bolded, and with six histidine codons). PCR was performed as follows: 0.8 μM primers, 200 μM dNTPs, 4 mM $MgSO_4$, 10% DMSO, 0.025 U of Pfu DNA Polymerase (Fermentas, USA) and 1 ng template per μL of reaction mixture; cycling parameters: denaturation at 95 °C for 3 min; following 28 cycles of denaturation at 95 °C for 50 s, annealing at 65 °C for 45 s, extension at 72 °C for 2 min, and final extension at 72 °C for 5 min. PCR product was digested with EcoRI and HindIII, ligated into pET-15b vector (creating pET-15bscL) and cloned into *E. coli* XL1-Blue. Plasmids pET-15bscL carrying correct *scL* sequence and pANT849pWB19N isolated from methylation deficient host (*E. coli* GM119) were EcoRI/HindIII digested. After digestion of pANT849pWB19 two fragments were obtained, 2.9 kb