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Review

Recent advances and applications of the lipolytic activity of *Carica papaya* latex

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ABSTRACT

The lipolytic enzymes of *Carica papaya* have been the subjects of a growing research activity in the last years due to the structural characteristics, substrate specificity over lipases from different sources and the wide availability from the large worldwide figures of papaya agro-waste. This review attempts to present an inclusive discussion of the recent advances of these lipolytic enzymes starting from findings related to their structure and mechanism and later examining the most employed pretreatments and the general optimized parameters for its use. Furthermore, individual sections comprising applications in glycerolipid modification, catalysis for the synthesis of non-glycerolipid products and the kinetic resolution of racemic compounds are discussed. The attractive features of CPL cited in this literature survey serve as an invitation for other research groups interested in the development of new and sustainable biochemical technologies.

Contents

1. Introduction

Lipases, also known as triacylglycerol ester hydrolases (EC 3.1.1.3), are one of the most versatile biocatalyst with a remarkable ability to achieve a wide range of bioconversion reactions using a variety of substrates. Moreover, lipases possess the unique property of working at a lipid/water interface mainly in organic media [1]. In most instances, commercial lipases are generally produced from animals or microorganisms [2–4]. Nonetheless, most of plant lipases are relatively inexpensive due to their wide availability from natural sources. As a result, plant lipases are generally more accepted for food or medicinal applications. However, the major impediment for its implementation at large scale is the low contents of enzyme in the postgermination seeds, bran portion of the grain and wheat gem. Noticeably, Caricaceae or Euphorbiceae overcome this disadvantage as their enzymes are available in large amounts stored in their latex [5,6]. In this context, the lipolytic complex of enzymes present in *Carica papaya* which, otherwise stated, are referred to as CPL when they are found in the crude latex without pretreatment or pCPL when they are in a crude lipase preparation, hold several advantages over their microbial and animal counterparts such as: (i) good stability to a wide range of pH, temperature, organic solvents and to the presence of other catalysts [7]; (ii) relatively inexpensive, e.g. the price is approximately

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about one third that of crude *Candida rugosa* lipase (CRL) [8,9]; (iii) can be considered "self-immobilized" enzymes since they are naturally bound to a non-water soluble matrix and thus, do not require further support and can be both recovered and reused [7]; (iv) the active sites do not require interfacial activation prompted by detergents such as the pancreatic lipase [4,10]; (v) the regio-, stereo-, typo- and substrate selectivities offer high versatility in diverse biochemical reactions; (vi) can be sustainably collected from the industrial papaya agro-waste of sick and unripe fruits [11–13].

The earliest report on papain lipases dates from 1925 [14], but it was not until 1991 that the first hydrolytic activity on various triacylglycerols (TAGs) was systematically evaluated [10]. From that moment on, *C. papaya* as source of lipases has been subject of a growing interest as a catalyzing tool in many biotechnological procedures, namely hydrolysis and various reverse reactions such as esterification, transesterification and aminolysis with reduced side products, lowered waste treatments and the tolerance to various reaction conditions [15]. Consequently, a large quantity of scientific research is constantly published and only a few specialized summaries are available. Owing to this fact, the scope of this review is to provide a broad insight of the recent advances in the knowledge of the lipolytic enzymes present in *C. papaya* while focusing mainly in the literature survey dated back to 2006 onwards and not covered in the previous review [16].

2. Structure and mechanism of the lipolytic enzymes of *C. papaya*

To date, some relevant enzymes have been isolated and characterized from *C. papaya* [17–19], among them papain is perhaps the most widely applied in the biochemical industry [20–22]. However, general biochemical research carried out with lipases derived from plant sources has been accomplished only on non-pure catalysts aside of a few exceptions [5]. In the case of the enzymes of *C. papaya*, a recent screening of its non-water soluble latex reported non-proteic molecular species such as saturated and unsaturated fatty acids, tocopherols, tocotrienols, triterpenic alcohols, sterols and the possible presence of short polyisoprene chains covalently bonded to phospholipid molecules forming a polymeric matrix [23]. When linked, the aforementioned species may provide a role of colloidal stability, which makes the purification of the lipolytic enzymes present in the latex hard to achieve with common separation techniques [19,24].

To the best of our knowledge, only three proteins with lipolytic properties present in the latex of *C. papaya* have been characterized through the aid of the recent sequencing of the *C. papaya* genome [25], although without being purified to homogeneity. The first of them was a GDSL-motif carboxylester hydrolase (CpEst) whose activity was found responsible for the hydrolysis of tributyrin and vinyl esters tested during the analysis. However, CpEst did not show a considerable specific activity (SA) towards long chain and medium chain TAGs, in contrast to the whole latex activity, acting as an esterase rather than a true lipase, which strongly suggested that total lipolytic activity in the crude latex could be attributed to more than one enzyme [26]. In a related study, washing the latex with organic solvents enhanced the lipolytic activity of *Vasconcellea heilbornii*, a close genetic relative of *C. papaya* that exhibits similar catalytic features [27,28]. The resulting protein-rich extract enabled the identification of a putative homologous lipase (CpLip1) that is also likely to code for the *C. papaya* protein. CpLip1 was identified as a member of the castor bean acid lipase structural family and showed SA towards both short and long TAGs [24]. In addition, the previously observed enzyme activity with phosphatidylcholine [7] can account for the recent partial purification of phospholipase D (CpPLD1) from *C. papaya* latex [29].

In regard of the catalytic machinery of the interfacial enzymes CpLip1 and CpEst, protein sequences showed that both share a catalytic triad which is similar to that of serine proteases [30,31]: a nucleophilic serine (Ser) residue activated by a hydrogen bond in relay with histidine (His) and aspartate (Asp) in addition to a relatively hydrophilic oxyanion hole that forms hydrogen bonds to the tetrahedral intermediate. The active site varies in the amino acids location in the protein [32,33]. As a result, a major difference between the two enzymes lies on the sequence that forms a "lid" of surface loop that surrounds the catalytic Ser that needs to undergo a conformational change before accessing the whole active domain [24,26]. On the other hand, the putative structure model of CpEst suggested that the catalytic triad is exposed at the surface of the molecule without a "lid" domain and a binding site for long chain fatty acids [26]. The catalytic mechanism in both enzymes includes an acylation step where the imidazole moiety of His withdraws a proton from Ser to enhance its nucleophilic attack at the carbonyl carbon. Furthermore, the imidazolium donates the proton to the leaving group oxygen from the tetrahedral intermediate and forms the acyl-enzyme intermediate. The deacylation step involves the ester or ester derivative hydrolysis via water or an organic base through a second tetrahedral intermediate (Scheme 1) [33–35].

3. Optimization of lipase biocatalyst

Taking advantage of the high stability among many proteases [7,37], the lipolytic moiety of *C. papaya* can be conveniently stored in the crude papain powder from papaya latex (CPL), which is also the commercial name given to the spray-dried latex (ref. P3375, *Sigma*). Papaya latex can alternatively be obtained directly from *C. papaya* or its relatives [38,39]. Although the level of proteins depends on papaya maturity and varies in the fruit, seed, leaf and root, lipase activity has only been reported in the first three papaya parts [40–42]. However, the latex is generally obtained by making a longitudinal incision on the epidermis of the unripe fruit and later stored at low temperatures [43,44]. Notably, a 90% of the original lipolytic activity may be preserved through a 40-week storage at 4 °C [7]. When the collected latex is dried and roughly crushed, it often receives the name P_3 in literature.

3.1. Catalyst pretreatments and operational stability

Although P_3 and commercial crude papain latex have exhibited fair lipolytic activity even without preliminary lipase purification in lipid modification [45,46], some articles have reported low or null reaction yields in other lipase applications [47–50]. As a result, some pretreatments have been developed to partially purify CPL and P_3 and to increase the enzyme affinity towards substrate. Some of the pretreatments enable the reutilization of the enzymatic complex in consecutive cycles maintaining a high catalytic activity. As the commercially purified papain protease from Sigma and the water soluble portion of the papaya latex were unable to catalyze lipase related reactions [8,37,46,49], distilled and deionized water are often used to wash-off soluble proteases present in the latex by centrifugation. The precipitate is then collected and lyophilized, resulting in a mass recovery ranging from 15 to 35% based on weight of the crude papain [7,34,51–53]. Additionally, treatments with proteases XXV and proteinase K have been used to reduce the resultant mass of CPL preparation by partially solubilizing its powder without noticeably losing its activity [7]. Aside from the common procedure, delipidation of pCPL powder with acetone markedly increased its SA in relation to the common extract preparation [41]. Other reports include a six-fold increase of CPL activity in methanolysis by pre-washing the enzymes with isopropanol, but not with *n*-propanol [43]. This observation reinforces the previous