RAPID COMMUNICATION

Cutinase—A New Tool for Biomodification of Synthetic Fibers

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INTRODUCTION

Synthetic fibers represent almost 50% of the worldwide market of textile fibers. Major characteristics of synthetic fibers are their hydrophobicity and low reactivity with most common chemical agents.[1] The low hydrophobicity makes those fibers less suitable to be in contact with the human skin, and the low reactivity makes the fiber unsuitable to act as carrier to other chemical finishing agents. Strong alkaline treatments can improve hydrophilicity and chemical reactivity of synthetic fibers but the treatment extension is hard to control, leading to unacceptable levels of strength loss.[1] Various attempts to overcome these difficulties were done before, using esterase enzymes to modify poly(ethyleneterephthalate),[2] and nitrilases to modify polyacrilonitrile fibers.[3]

Cutinase was chosen for the modification of synthetic fibers because it is described as esterase that degrades cutin, a structural polyester of plants.[4] Cutinase is a serine hydrolase with low specificity that is known to hydrolyze *p*-nitrophenyl esters and soluble and insoluble triglycerides.[4]

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In this article, we describe the use of *Fusarium solani pisi* cutinase to modify the surface of textile synthetic fibers like polyester, polyamide 6.6, and acrylics. In polyester, the cutinase hydrolysis yields terephthalic acid and etylenoglicol. In polyamide, the cutinase hydrolysis yields hexamethylenediamine and adipic acid. The cutinase hydrolysis of acrylics (constituted by polyacrylonitrile and $\approx$ 7% of vinyl acetate as comonomer) yields acetic acid, leaving vinyl alcohol at the fiber surface.

EXPERIMENTAL

In this work we used a wild-type cutinase from *Fusarium solani pisi*, that was overproduced by the *Saccharomyces cerevisiae* SU50 strain transformed with the expression vector pUR7320, as described.[5] The cutinase was obtained in the extracellular medium in a crude form with a purification degree between 50 and 70% in relation to total protein in the culture medium as previously described[6] and used after cell separation by centrifugation without any further purification. The crude preparation had no protease activity measured as azocasein.

The activity of cutinase was based in the conversion of *p*-nitrophenylpalmitate (pNPP) into *p*-nitrophenol[7] and followed spectrophotometrically at 405 nm. The activity

Table 1. Enzymatic Activity on Synthetic Heterogeneous Substrate

Substrates	Activity (U*)
p- nitrophenyl palmitate (pNPP)	1
Polyamide	$\sim 2.3 \times 10^{-3}$
Polyester	$\sim 2.5 \times 10^{-5}$
Acrylic	$\sim 4 \times 10^{-4}$

* 1 Unit is the conversion of 1 micromole of substrate per minute. See Experimental section for details.

in polyamide substrate was determined by the production of hexamethylenediamine quantified with the TNBS method by Morçöl et al.[8] in which the primary amines react with the sodium salt of 2,4,6-trinitrobenzenesulfonic acid (TNBS). A protein pre-elimination step was performed with trichloroacetic acetic before the amine quantification. The activity in polyester substrates was determined by following the production of terephthalic acid in the bath solutions treatment[2]; the absorbance was read at 240 nm. The activity in acrylic substrates was determined by detection of acetic acid, using kit no. 0148261 from Boheringer. The protein concentration was measured in the bath solution using the Bradford method, at 595 nm.[9] The results shown in Table 1 compare the different substrates.

All activity and treatment measurements were done in phosphate buffer (50 mM) at 37 °C and in a time range of 4 min for pNPP and several hours for fiber substrates. Activities were calculated in the linear area of substrate conversion. Control experiments were done at time zero with enzyme and buffer solution.

The treatment of polyamide 6.6, polyester, and acrylics with cutinase enzyme was done in an Ahiba pot reactor with mild agitation at 30 °C using conditions described in Figure 1. All fabrics were rinsed thoroughly with 2% sodium carbonate solution for protein removal (with negative staining levels with Coomassie Brilliant Blue G dye[9]), then dried and used for reactive dyeing. The conditions for dyeing were: Polyamide (2% o.w.f. of reactive red 66; 50 gL^{-1} of NaCl; temp. 60 °C; during 90 min.; reductive washing with sodium hydrosulfite (2 gL^{-1})); Polyester (1% o.w.f. of reactive black 5; 50 gL^{-1} of NaCl and 10 gL^{-1} of Na_2CO_3; temp. 60 °C; during 90 min.; reductive washing with sodium hydrosulfite (2 gL^{-1})); Acrylic (2% o.w.f. of reactive blue 19; 50 g L^{-1} of NaCl and 10 g L^{-1} of Na_2CO_3; temp. 70 °C; during 90 min.). Mild postwashing with a reductive agent for polyamide and polyester was performed to remove dye excess that was not covalently bound.

Sensitivity of Reactive Dyeing to Identify the Superficial Amino and Hydroxyl Fiber Groups

Reactive dye molecules are composed of a chromophoric structure linked with a reactive group.[10] The reactive vinylsulphonic groups (in CI Reactive Blue 18 and CI Reactive Black 5) react specifically with hydroxyl groups under alkaline conditions, and the bromoacrylamide (in CI Reactive Red 66) groups react specifically with amino groups under slightly acidic conditions.[10] We have adapted the use of cotton reactive dyes and wool reactive dyes to track, respectively, hydroxyl and amino groups at the surface of synthetic fibers. Those dyes have not been designed to have affinity for synthetic fibers; therefore, only the dyes that react with hydroxyl and amino groups will stay on the fiber. This is a direct method to quantify hydroxyl and amino groups at the fiber surface, and it is very sensitive due to large extension coefficients of the chromophoric structures. Just a few amino or hydroxyl superficial groups at the fibers will produce visible differences measured by reflectance spectrometry as K/S units.

RESULTS AND DISCUSSION

The relative activities of cutinase over a soluble substrate (pNPP) and the fiber substrates are shown in Table 1. One activity unit over soluble substrate pNPP yields lower activities over fiber substrates in the range of 10^{-3} at 10^{-5}. The detection of soluble reaction products for the fibers by cutinase action can only be measured after several hours, while for pNPP it can be seen in the range of a few minutes. All those synthetic fiber substrates have closed packed structure,[1] and it can be expected that a big enzyme molecule (> 24 kDa) will modify only the surface of synthetic fibers in a slow kinetic process. As described in the Introduction, cutinase was chosen for fiber modification due to its hydrophobic nature and because it was designed by nature to act on cutin plant polyesters. Most of its outer amino acid residues closed to the active site are hydrophobic, possibly enhancing better enzyme–fiber interactions.[11]

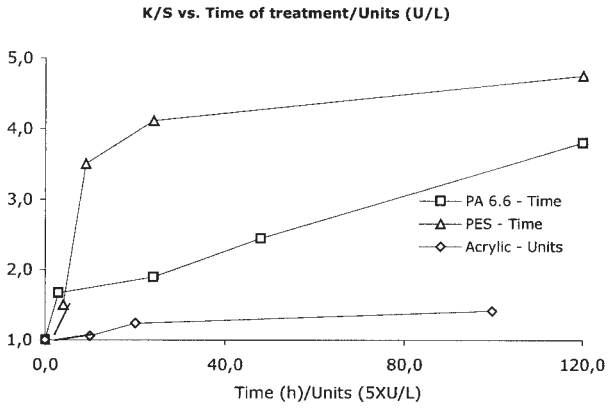

Figure 1. K/S (% of increase towards control samples) of polyamide (2.42$U_{cutinase}$/g of PA), polyester (0.013$U_{cutinase}$/g of PES), and acrylic (treated for 90 h) samples. See Experimental section for dyeing details.

The action of esterases on PET was described before,[2] and the action of a cutinase over PET is expected since this enzyme was designed by nature to act on polyesters. We report here for the first time the action of cutinase on vinyl acetate (comonomer in acrylic commercial fiber), and this fact is not unexpected since cutinase is described as an nonspecific esterase acting on several soluble and insoluble esters[4]. Surprisingly we found that cutinase could hydrolyze amide bonds of polyamide fibers. Apparently, there are a lot of amidases that share the same catalytic triad with esterase enzymes.[4] Also, the hydrophobic nature of this fiber[1] might enhance the polyamide chain docking in the exposed cutinase active site[11] and therefore the hydrolysis of polyamide. However, the mechanism of this reaction is not completely clear to us and further studies are needed.

We further tracked the changes at the fiber surface by reactive dyeing after enzymatic action (Fig. 1). The enzyme action leaves hydroxyl groups (in the case of polyester and in the comonomer of acrylic) and amino groups (in the case of polyamide). In Figure 1 we see the increase of amino and hydroxyl groups at the fiber surface, formed due to cutinase action. The reaction is somewhat slow (reaction times higher than 24 h) for a possible industrial application of this technology. The modification of acrylic fibers with cutinase yields the lowest increase of color, because very few units of vinyl acetate are present (approximately 1 in 20 units) to be modified by cutinase.

Cutinase has a higher activity on polyamide than on polyester (Table 1) but the reverse can be seen on the formation of end groups at the fiber surface (Fig. 1). The activity on polyamide and on polyester was measured as soluble amino and carboxylic groups of monomers and oligomers produced by enzymatic action. The relation between produced end groups on the fiber and soluble reaction products in solution depends on the mode of action of the enzyme and also on the solubility of the monomers/oligomers produced. Solubility data indicate the adipic acid is more soluble by a factor of 5 than terephthalic acid, as well as that oligomers of polyester are less soluble than the short oligomers of polyamide 6.6. Therefore, more free ends are at the fiber surface of polyesters, yielding high color intensities (Fig. 1). Since we have used the same enzyme, the solubility difference between oligomers of polyester and polyamide seems to better explain the relation between end groups on the fiber and soluble reaction products.

The results of this study show the ability of cutinase to modify synthetic fibers containing ester or amide groups. This enzyme has an "hydrophobic head" nearby the active site; therefore, it constitutes an ideal tool for the modification of hydrophobic fiber surfaces.[11]

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