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Enzymatic lipid removal from surfaces—lipid desorption by a pH-induced "electrostatic explosion"

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Abstract

Removal of lipidic molecules from surfaces can be accomplished using detergents containing lipases. Surface cleaning is usually performed under alkaline conditions due to increased solubility of the hydrolysis products, especially free fatty acids. This paper shows that removal of a triacylglycerol film from a surface can be dramatically enhanced in a sequential system where pH is shifted to alkaline conditions after an initial lipolytic reaction period at or below neutral pH. Data from three different biophysical techniques, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), quartz crystal microbalance with dissipation monitoring (QCM-D), and total internal reflection fluorescence spectroscopy (TIRF) clearly show the effects of such cleaning procedure. Initially the reaction is carried out at pH below the pK_a value of the fatty acids formed upon triacylglycerol hydrolysis, and the protonated fatty acids accumulate in the film. The mechanism of lipid removal, induced by increasing pH to a value above the fatty acid pK_a, is explained by a burst caused by electrostatic repulsion between rapidly ionised fatty acids, i.e. by an "electrostatic explosion". Performing the initial hydrolysis at pH 6 and the subsequent rinse at pH 10, using triolein as model substrate, lipid removal from surfaces by both commercial detergent lipases and non-commercial lipases was significantly improved compared to a reaction at constant pH 10.

Keywords: Lipase activity; Lipid removal; Lipase adsorption; Triacylglycerol; ATR-FTIR; QCM-D; TIRF

1. Introduction

A major problem when cleaning surfaces is the removal of adsorbed lipid deposits, which often contain oily, longchained, and water-insoluble triacylglycerols. Detergent formulations usually contain lipolytic enzymes (lipases, formally triacylglycerol lipases, E.C. 3.1.1.3), which degrade triacylglycerols into free fatty acids, di- and mono-acylglycerols, and possibly glycerol. These hydrolysis components, especially the fatty acids, are more water soluble compared to triacylglycerols (Fujii et al., 1986). With aid from

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detergent components and their ability to form water soluble aggregates, fatty acids may solubilize into the aqueous washing solution and thereby be released from the surface. Lipases are essential in detergent formulations when cleaning at low temperatures (Dambmann et al., 1971).

The lipase from *Thermomyces lanuginosus*, formerly *Humicola lanuginosa*, was the first major commercial lipase for detergents. The *T. lanuginosus* lipase (TLL) is produced and sold by Novo (now Novozymes, Denmark) since 1989 as lipolase (Aaslyng et al., 1991). Though lipolase is stable in detergent formulations, at high pH, and at high temperatures, 2–3 wash cycles (which includes drying of the textile) are needed to obtain a significant removal of lipidic stains (Flipsen et al., 1998; Aaslyng et al., 1991).

One suggestion explaining this lack of "first wash activity" by lipolase is that the main lipolytic activity occurs during the drying process (Flipsen et al., 1998; Aaslyng et al., 1991). Triacylglycerol removal is therefore only achieved after the second wash when the detergent solution and the mechanical stress can remove the hydrolysis products produced during the first drying (Aaslyng et al., 1991).

We have outlined in a previous paper an alternative explanation, suggesting that the formation rate of ionised fatty acids (as a result of lipase activity) must exceed a certain threshold in order to obtain lipid removal (Snabe and Petersen, 2003). The mechanism is explained by an equilibrium between hydrolysis products being incorporated into the substrate film or forming micelle-like aggregates which can be solubilized and ejected into the aqueous solution. Interestingly, it was found that the equilibrium favours lipid removal only under alkaline and Ca^{2+}-free conditions and when the lipolytic rate is above a certain threshold. Consequently it was proposed that the equilibrium of lipid removal is controlled by the formation rate of ionised fatty acids.

Flipsen et al. (1998) reported an empirical study of the removal of triolein soil from textile using lipases. It was found that the triacylglycerol lipase cutinase displayed significant lipid removal (20%) at constant pH 9—thus cutinase can be termed a "first-wash lipase". However, in a sequential system where the initial incubation at pH 9 was followed by a subsequent wash at pH 10.5–11, the removal of lipid increased to 35%. The improvement was explained in terms of increasing lipid solubility in aqueous environment at increasing pH values.

The present paper shows that significant lipid removal from surfaces by a large range of lipases can be induced by carrying out the washing procedure at two different pH values. The washing process is preferably started below the pK_a value of the released fatty acids. Hydrolysis should then be allowed to progress for some time allowing accumulation of protonated fatty acids in the lipid film. Subsequently the pH of the solution in contact with the surface to be cleaned is raised above the pK_a value of the fatty acid molecules. This procedure results in a significantly improved lipid removal (up to 50%) compared to the usual procedure performed under constant alkaline conditions. Using the longchained triolein as substrate, results are presented with the native *T. lanuginosus* lipase (TLL) and engineered TLL variants, as well as lipases from *Rhizomucor miehei*, *Pseudomonas cepacia*, and *Fusarium solani* pisi (cutinase).

2. Materials and methods

2.1. Materials

Triolein 99% (Fluka 92859, lot. 2045347) was used as substrate. Seven triacylglycerol lipases (E.C.3.1.1.3) were used: *T. lanuginosa* lipase (TLL) and engineered TLL variants, i.e. lipolase, lipoprime, lipex, and an inactive TLL (Novozymes, Denmark), as well as lipases from *R. miehei* (Sigma L9031, lot 32K2604) *Ps. cepacia* (Sigma L9156, lot 85H2604), and *F. solani* pisi (cutinase) produced in our laboratory (Petersen et al., 1998). Protein concentrations were determined using UV_{280} absorption and the respective extinction coefficients at 280 nm. Buffer of $3\times$ 25 mM citrate–Tris–glycine (25 mM of each component) with or without 10 mM $CaCl_2$ was prepared from citrate trisodium dihydrate 99% (Sigma S4641,19H0717), glycine 99.5% (Acros Organics, 220910010/200-272-2), Tris 99.9% (Applichem A1086,C2101), and calcium chloride dihydrate 99.5% (Merck 1.02382, lot TA401282). pH was adjusted with 3 M sodium hydroxide or hydrochloric acid and filtered through a 0.20 μm filter before use (Sartorius Minisart 16534 K). Reagents for preparation and triolein coating of TIRF quartz slide surfaces: dichlorodimethylsilane (Fluka 40150,