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Effects of urea and sodium dodecylsulphate on the stability profile of *Thermomyces lanuginosus* lipase

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ABSTRACT:The thermostability property of Thermomyces lanuginosus lipase (TLL) has been demonstrated but its resistance to protein denaturants has not been fully characterized. Therefore, this study investigated the effects of protein denaturants, urea, and sodium dodecyl sulphate (SDS), on TLL activity. Reaction mixtures containing 0.1 M Glycine-NaOH buffer, 0.001 µM TLL, and appropriate concentrations of protein denaturants were pre-incubated at 37 oC for 10 minutes. Reactions were initiated by the addition of appropriate concentration of substrate, para-nitrophenyl dodecanoate (pNPD), for a specific time interval and were terminated by the addition of 0.5 M TCA and 0.5 M NaOH. TLL activity was determined spectrophotometrically at 405 nm by monitoring the rate of hydrolysis of pNPD against a blank of the buffered substrate. TLL was active over a pH range of 3.0 to 9.0 and its activity was optimal at pH 9.0. However, TLL activity dropped by 41% at pH 12.0. TLL activity increased progressively with increase in temperature from 20 °C to 60 °C. Between 70 and 100 °C, a slight decrease in TLL activity was observed as compared to the activity at 60 °C. TLL activity was stable at pre-incubation temperatures ranging from 30 °C to 80 °C. Urea at 0.1 – 4.0 mM increased TLL activity. SDS at 0.5 – 4.0 mM increased TLL activity in a concentration-dependent manner with optimal activity obtained at 4.0 mM. Urea at 0.1, 1.0, 2.0 and 4.0 mM decreased maximum reaction rate (Vmax), catalytic constant (Kcat) and Michaelis constant (Km) of TLL, while SDS at 0.1 mM decreased Vmax, Kcat and Km. 1.0 mM SDS did not affect Vmax and Kcat of TLL, but reduced Km by 50%. At 2.0 mM SDS, Vmax, and Kcat increased by 43% while Km reduced. However, 4.0 mM SDS reduced Vmax, Kcat, and Km of TLL. In conclusion, findings from this study suggest that TLL partially and strongly resists denaturation by optimal concentrations of urea and SDS, respectively.

Keywords: Thermomyces lanuginosus lipase, thermostability, protein denaturants

Introduction

Lipases (triacylglycerol acyl hydrolase, EC 3.1.1.3) are special kinds of esterases that catalyze the hydrolysis of triacylglycerols to give free fatty acids, diacylglycerols, monoacylglycerols, and glycerol (Fernandez-Lafuente, 2010). Lipases are ubiquitous enzymes found in animals, plants, fungi and bacteria, and are of considerable physiological significance and with industrial potentials (Khan *et al*., 2017). In

contrast to esterases, lipases are activated only when adsorbed to an oil-water interface and do not hydrolyze dissolved substrates in the bulk fluid (Khan *et al*. 2017).

Lipases have a property in common: in homogenous medium, they have their active center secluded from the medium by a polypeptide chain called lid (Figure 1) (Barbe *et al*., 2011). The lid is an alpha-helical mobile surface loop consisting of 86–93 amino acids that covers the active site, which consists of a typical serine-histidine-aspartate catalytic triad (Khan *et al*., 2017).

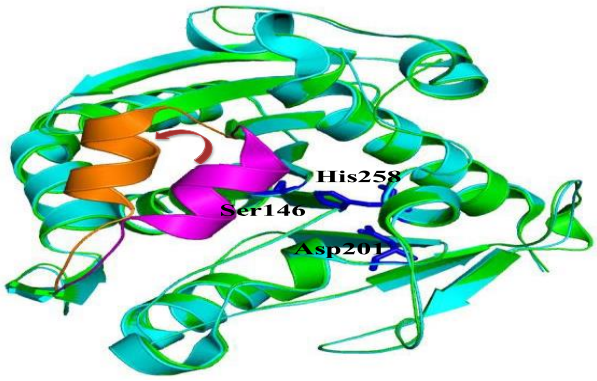

Figure 1: Superimposition of Close (green) and Open (cyan) Conformations of *Thermomyces lanuginosus* Lipase. The close lid, open lid and catalytic triads were highlighted by magenta, orange and blue colours, respectively. Source: Khan *et al*. (2017).

The lid usually takes a closed conformation in the inactive form of the enzyme (Figure 2), when lipase is however adsorbed to a water-lipid interface, the lid is displaced and the active site becomes accessible to a substrate (open conformation) (Fernandez-Lafuente, 2010).

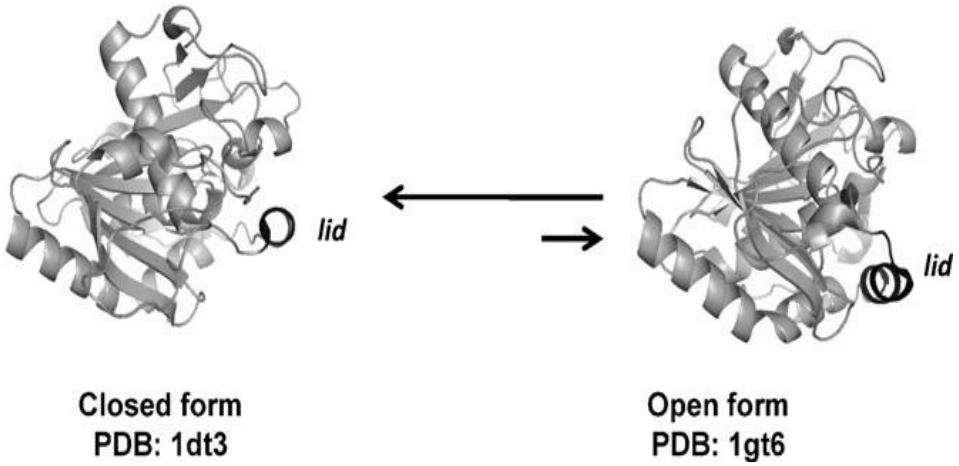

Figure 2: 3D Structure of Open and Closed Forms of *Thermomyces lanuginosus* Lipase Active-site Lid. The 3D structure was obtained from the Protein Data Bank (PDB) using Pymol vs. 0.99.
Source: Fernandez-Lafuente (2010).

Thermomyces lanuginosus lipase (TLL) is one of the most studied lipases with thermostable characteristics (Skjold-Jorgensen et al., 2014). The high activity, stability, and wide substrate specificity of TLL have permitted its use in pharmaceutical production, biodiesel, detergent, and food manufacturing (Fjerbaek et al., 2009).

Urea and Sodium dodecylsulphate (SDS) are well-known protein denaturants (Wang et al., 2013; Monhemi et al., 2014). While enzyme denaturation by urea has been likened to what is observed in

thermal denaturation above protein's melting temperature (Tm) of 75 °C, SDS unfolds proteins by binding unspecifically to their surface in the process causing them to lose their functions (Salameh and Wiegel, 2010; Canchi and Garcia, 2013). For TLL to maintain its industrial relevance there is a need for it to be stable and effective in complex mixtures of compounds such as organic compounds and anionic detergent (Fernandez-Lafuente, 2010). The thermostability of Thermomyces lanuginosus lipase (TLL) has been established but its resistance to protein denaturants has not been fully characterized. Therefore, this study investigated the effects of protein denaturants, urea, and sodium dodecyl sulphate (SDS), on TLL activity.

Materials and Methods

Materials

Para-nitrophenyl dodecanoate (pNPD) also known as 4-Nitrophenyl ester C18H27NO4 and Sodium Dodecylsulphate (Lauryl Sulphate) Sodium salt (C12H25O4SNa) were products of Sigma - Aldrich Co, St. Louis, USA. Urea (NH2CONH2) is a product of NAAFCO, Scientific Supplies Ltd. New York. All reagents were purchased from Fisher Scientific, UK, and were of analytical grade. Homogenous Thermomyces lanuginosus lipases were synthesized and purified at the Institute of Molecular Cell and Systems Biology, University of Glasgow, Bower Building, Glasgow G12 8QQ, Scotland, UK. 0.1 M Glycine-NaOH buffer was freshly prepared each week by addition of 0.751 g glycine in 100 ml distilled water to 40.0 g NaOH in 100 ml distilled water, the solution was mixed carefully and adjusted to pH 9.4. The standard substrate, para-nitrophenyl dodecanoate (pNPD) was prepared freshly daily in 100% ethanol as a 2.0 mM solution by the addition of 10 ml of 10 mM pNPD to 50 ml 100% ethanol. Thermomyces lanuginosus lipase was prepared in the reaction buffer as 0.001 µM solution and stored at -4 °C.

Determination of Thermomyces lanuginosus Lipase Activity

Thermomyces lanuginosus Lipase (TLL) activity was determined spectrophotometrically as previously described by Gopinath *et al*. (2005) with some modifications. Reaction mixtures containing 0.1 M Glycine-NaOH buffer, 0.001 µM TLL, and appropriate concentrations of protein denaturants were pre-incubated at 37 oC for 10 minutes. Reactions were initiated by the addition of appropriate concentration of pNPD, for specific time intervals and were terminated by the addition of 0.5 M TCA and 0.5 M NaOH. TLL hydrolyses the colourless synthetic substrate, para-nitrophenyl dodecanoate (pNPD), to produce a yellow-colored, para-nitrophenol (pNP). The amount of the para-nitrophenol (pNP) released from the hydrolysis of pNPD catalyzed by TLL according to Beer-Lambert's law was measured spectrophotometrically at 405 nm against a blank of the buffered substrate. All assays were carried out in triplicates.

Effect of pH on Thermomyces lanuginosus Lipase Activity

The effect of pH on Thermomyces lanuginosus lipase activity was determined as previously described by Fernandes *et al*. (2004) with some modifications. Reaction mixtures containing 0.1 M Glycine-NaOH buffer (pH 3 - 12) and 0.001 µM TLL were pre-incubated in a water bath at 37 oC for 10 minutes. Reactions were initiated by the addition of 0.08 mM pNPD and then incubated for 10minutes at 37 °C. The reaction was terminated by the addition of 0.5 M TCA and 0.5 M NaOH. Absorbance was read at 405 nm against a blank of the buffered substrate.

Effect of Temperature on Thermomyces lanuginosus Lipase Activity

The effect of temperature on Thermomyces lanuginosus lipase activity was determined as previously described by Fernandes *et al*. (2004) with some modifications. Reaction mixtures containing 0.1 M Glycine-NaOH buffer (pH 9.4) and 0.001 µM TLL were not initially pre-incubated. Reactions were initiated by the addition of 0.08 mM pNPD to the reaction mixtures and then incubated for 10 minutes at

different temperatures (20 - 100 °C). Reactions were then stopped by the addition of 0.5 M TCA and 0.5 M NaOH. Stability at high temperatures (30 – 80 °C) was measured using endpoint reactions in 0.1 M Glycine-NaOH buffer. Reaction mixtures containing 0.1 M Glycine-NaOH buffer (pH 9.4) and 0.001 µM TLL were pre-incubated in a water bath at varying temperatures (30, 35, 40, 45, 50, 60, 70, 80 °C) for 10 minutes and then placed on ice to cool. Reactions were initiated by the addition of 0.08 mM pNPD and then incubated for 10 minutes at 37 °C. Reactions were terminated by the addition of 0.5 M TCA and 0.5 M NaOH. Absorbance was read at 405 nm against a blank of the buffered substrate.

Effect of Urea and Sodium Dodecylsulphate on Thermomyces lanuginosus Lipase Activity

The effects of urea and SDS on Thermomyces lanuginosus lipase activity were determined spectrophotometrically at 405 nm as previously described by Salameh and Wiegel (2010) with modifications by using endpoint reactions in 0.1 M Glycine-NaOH buffer. Reaction mixtures containing 0.1 M Glycine-NaOH buffer (pH 9.4), with 0.1 – 4.0 mM urea/SDS and 0.001 µM TLL were pre-incubated in a water bath at 37 °C for 10 minutes. Reactions were initiated by the addition of 0.08 mM pNPD and then incubated at 37 °C for 10 minutes. Reactions were terminated by the addition of 0.5 M TCA and 0.5 M NaOH. Absorbance was read at 405 nm against a blank of the buffered substrate.

Kinetics of Thermomyces lanuginosus Lipase Catalysed Hydrolysis of para-Nitrophenyl Dodecanoate

The kinetics of Thermomyces lanuginosus Lipase (TLL) catalyzed hydrolysis of para-nitrophenyl dodecanoate was determined spectrophotometrically as previously described by Gopinath *et al*. (2005) with some modifications by the use of endpoint reactions in 0.1 M Glycine-NaOH buffer. Reaction mixtures containing 0.1 M Glycine-NaOH buffer (pH 9.4) and 0.001 µM TLL were pre-incubated in a water bath at 37 °C for 10 minutes. Reactions were initiated by the addition of varying concentrations of pNPD (0.01 – 0.50 mM) and then incubated at 37 °C for 10 minutes. Reactions were terminated by the addition of 0.2 ml of 0.5 M TCA and 0.25 ml of 0.5 M NaOH. Absorbance was read at 405 nm against a blank of the buffered substrate. The data were fitted to the Michaelis-Menten curve and Lineweaver-Burk plot using Microsoft Excel and kinetic constants (Vmax and Km) were obtained.

Kinetics of Thermomyces lanuginosus Lipase Catalysed Hydrolysis of para-Nitrophenyl Dodecanoate in the Presence of Urea/Sodium Dodecylsulphate

The effect of urea and SDS on the kinetic parameters of TLL were determined as previously described by Salameh and Wiegel (2010) with some modifications by the use of endpoint reactions in 0.1 M Glycine-NaOH buffer. The reaction mixtures containing 0.1 M Glycine-NaOH buffer (pH 9.4), varying concentrations of urea/SDS (0.1 – 4.0 mM), and 0.001µM TLL were pre-incubated in a water bath at 37°C for 10 minutes. Reactions were initiated by the addition of varying concentrations of pNPD (0.01 – 0.50 mM) and then incubated at 37°C for 10 minutes. Reactions were terminated by the addition of 0.2 ml of 0.5 M TCA and 0.25 ml of 0.5 M NaOH. Absorbance was read at 405 nm against a blank of the buffered substrate. The data were fitted to the Michaelis-Menten curve and Lineweaver-Burk plot using Microsoft Excel and kinetic constants (Vmax and Km) were obtained.

Results

Effect of pH on *Thermomyces lanuginosus* Lipase Activity

The effect of varying pH on Thermomyces lanuginosus lipase (TLL) catalyzed hydrolysis of para-nitrophenyl dodecanoate (pNDP) was investigated in this study. TLL was active over a pH range of 3.0 to 9.0 and its activity was optimal at pH 9.0 with 16% increase when compared to pH 3.0. However, TLL activity dropped by 41% at pH 12.0 when compared to pH 9.0 (Figure 3).

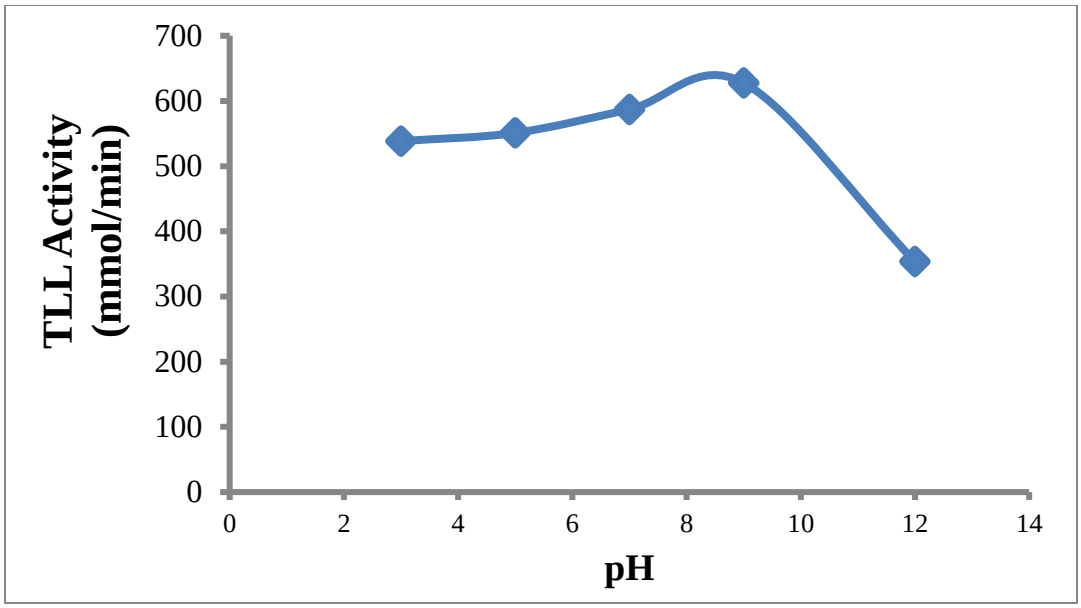

Figure 3: Effect of varying pH on *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

Effect of Temperature on *Thermomyces lanuginosus* Lipase Activity

The effects of varying assay temperatures (20 – 100 °C) and varying pre-incubation temperatures (30 – 80 °C) at assay temperature of 37 °C on TLL activity were investigated in this study. TLL activity increased progressively with an increase in temperature from 20 °C to 60 °C with 75% increase. Between 70 and 100 °C, a 21% decrease in TLL activity was observed as compared to the activity at 60 °C (Figure 4). TLL activity was stable at pre-incubation temperatures ranging from 30 °C to 80 °C with optimal stability at 60 °C (Figure 5).

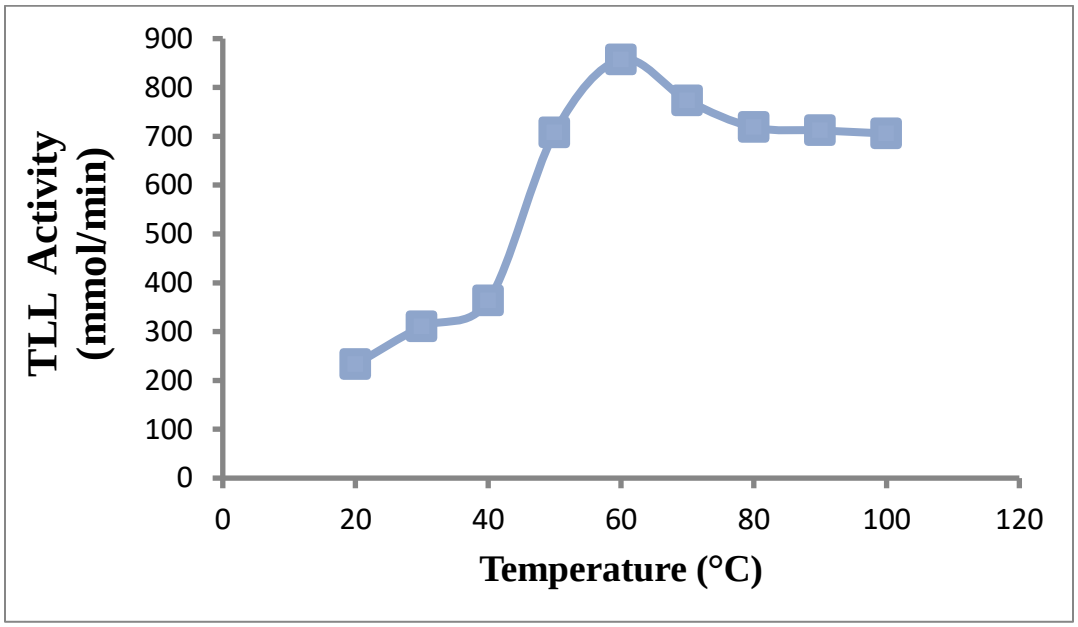

Figure 4: Effects of varying assay temperatures (20 – 100 °C) on *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

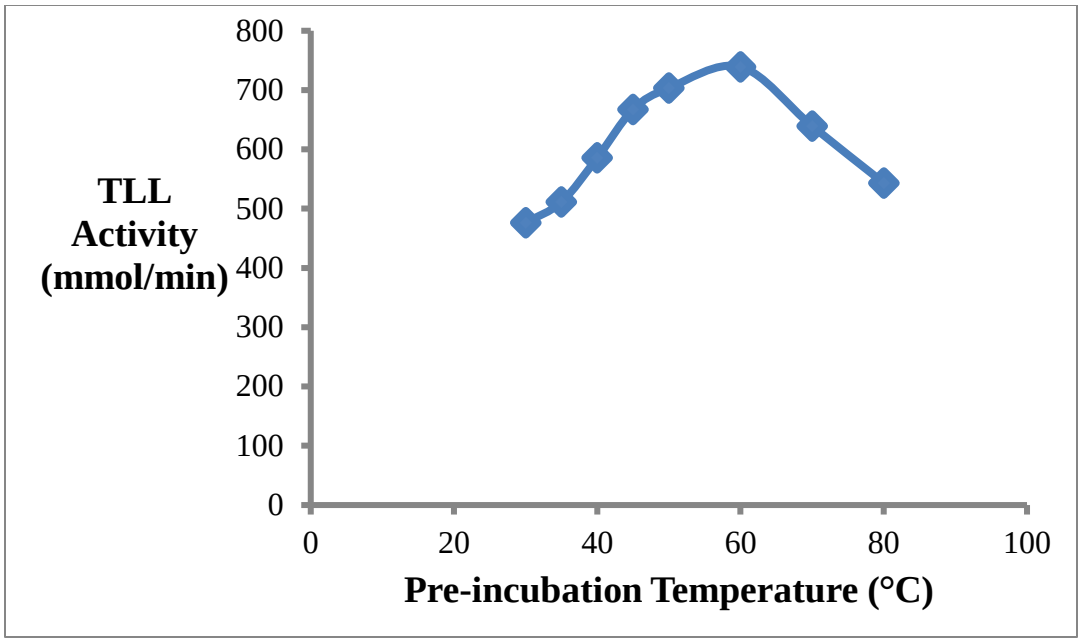

Figure 5: Effects of varying pre-incubation temperatures (30 – 80 °C) at assay temperature of 37 °C on *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

Effect of Urea and Sodium Dodecylsulphate on *Thermomyces lanuginosus* Lipase Activity

In this study, the modulatory effect of varying concentrations of urea and sodium dodecylsulphate (SDS) on TLL activity was examined in the presence of 0.08 mM pNPD using three independent assays. Urea at 0.1 – 4.0 mM increased TLL activity in a manner that is not concentration-dependent with optimal activity at 4.0 mM (Figure 6). At 0.1 mM SDS, TLL activity slightly increased. However, as SDS concentration increased from 0.5 to 4.0 mM, TLL activity increased in a concentration-dependent manner with 58% increase at 4.0 mM (Figure 7).

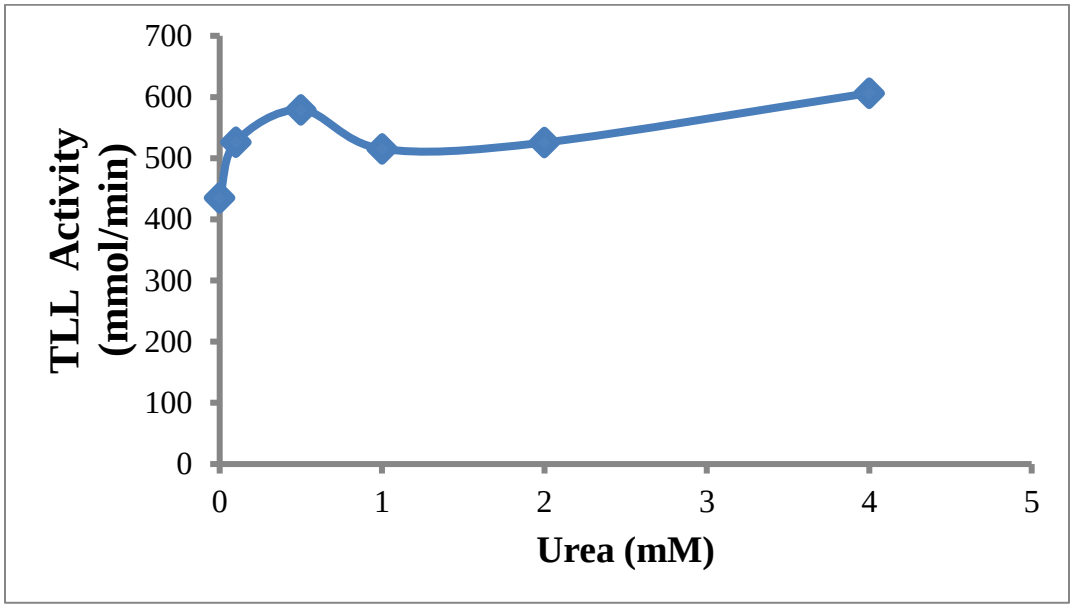

Figure 6: Effects of varying concentrations of urea on *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

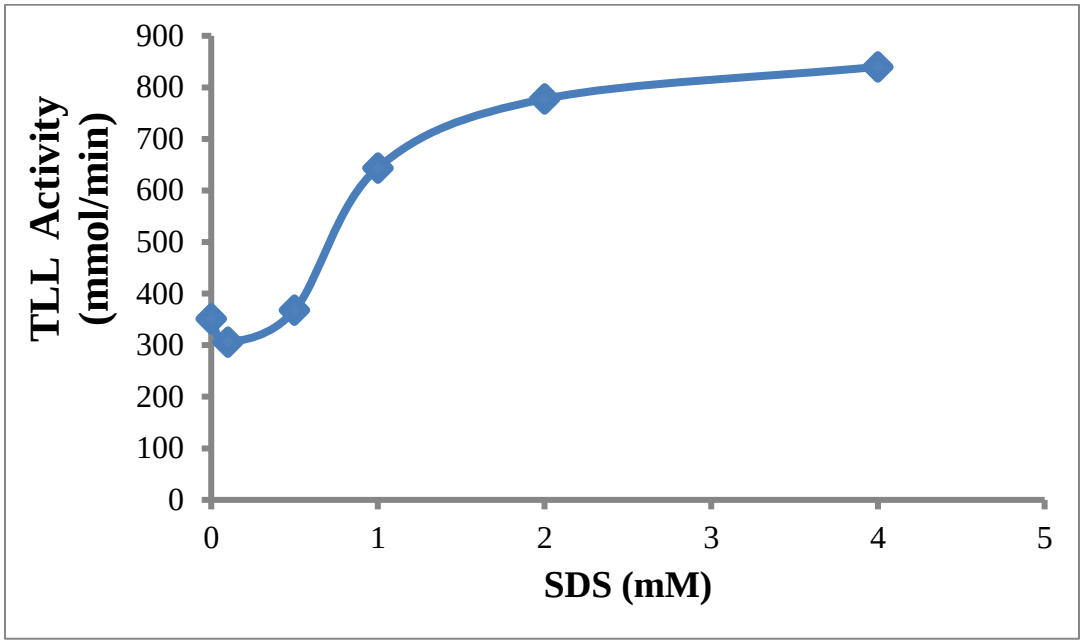

Figure 7: Effects of varying concentrations of sodium dodecyl sulfate on *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

Kinetics of *Thermomyces lanuginosus* Lipase Catalysed Hydrolysis of para-Nitrophenyl Dodecanoate

The substrate kinetics of TLL catalyzed hydrolysis of pNPD follows the classical Michealis-Menten hyperbola curve (Figure 8). From the Lineweaver-Burk plot, Vmax, Km, and Kcat were calculated as 10,000 mmol/min, 2.0 mm, and 18.9 x 104 sec-1, respectively (Figure 9).

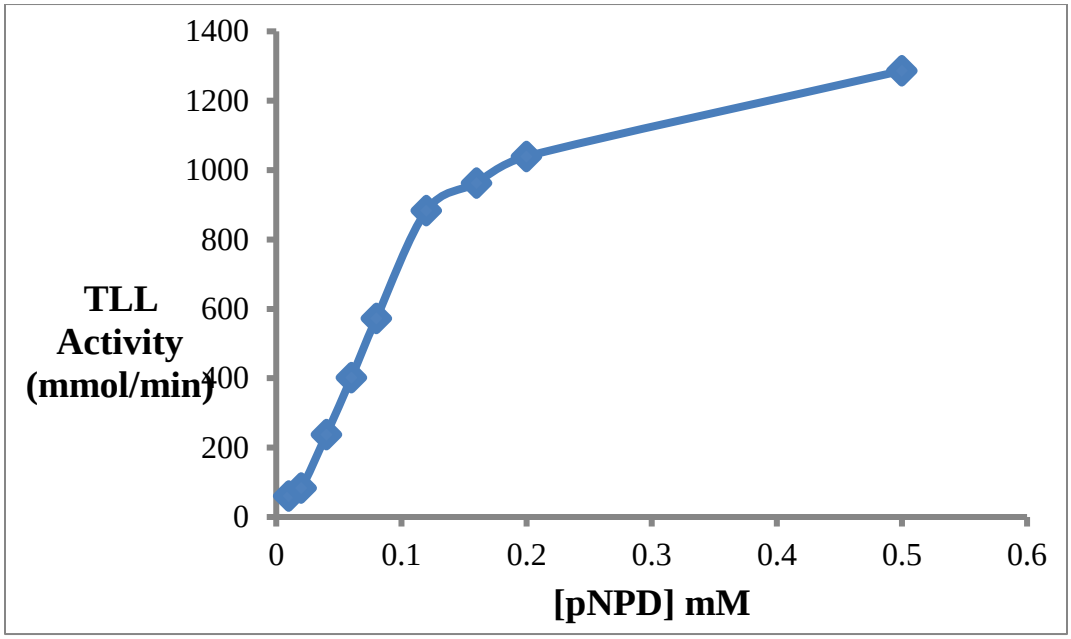

Figure 8: Michealis Menten curve of *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

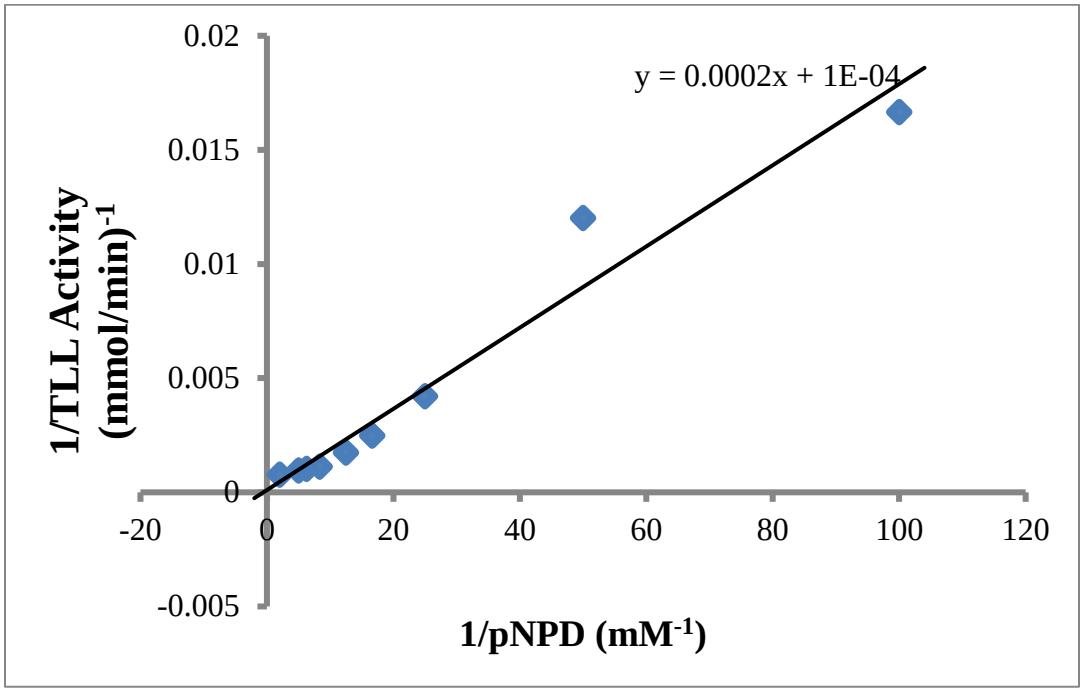

Figure 9: Lineweaver-Burk plot of *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

Kinetics of *Thermomyces lanuginosus* Lipase Catalysed Hydrolysis of para-Nitrophenyl Dodecanoate in the Presence of Urea

The substrate kinetics of TLL catalyzed hydrolysis of pNPD in the presence of urea follows the classical Michealis-Menten hyperbola curve (Figure 10). From the Lineweaver-Burk plot in Figure 11, Vmax, Km, and Kcat values were calculated as shown in Table 1. Urea at 0.1, 1.0, 2.0 and 4.0 mM decreased maximum reaction rate (Vmax), catalytic constant (Kcat) and Michaelis constant (Km) of TLL (Table 1). At 0.1 mM urea, the Vmax, Km, and Kcat decreased by 80%, 90% and 80%, respectively when compared with the absence of urea. In the presence of 1.0 and 2.0 mM urea, the Vmax and Kcat of TLL decreased by 83%, while Km decreased by 91 and 96% respectively. At 4.0 mM urea, the Vmax, Km, and Kcat decreased by 87%, 95% and 87%, respectively when compared with the absence of urea.

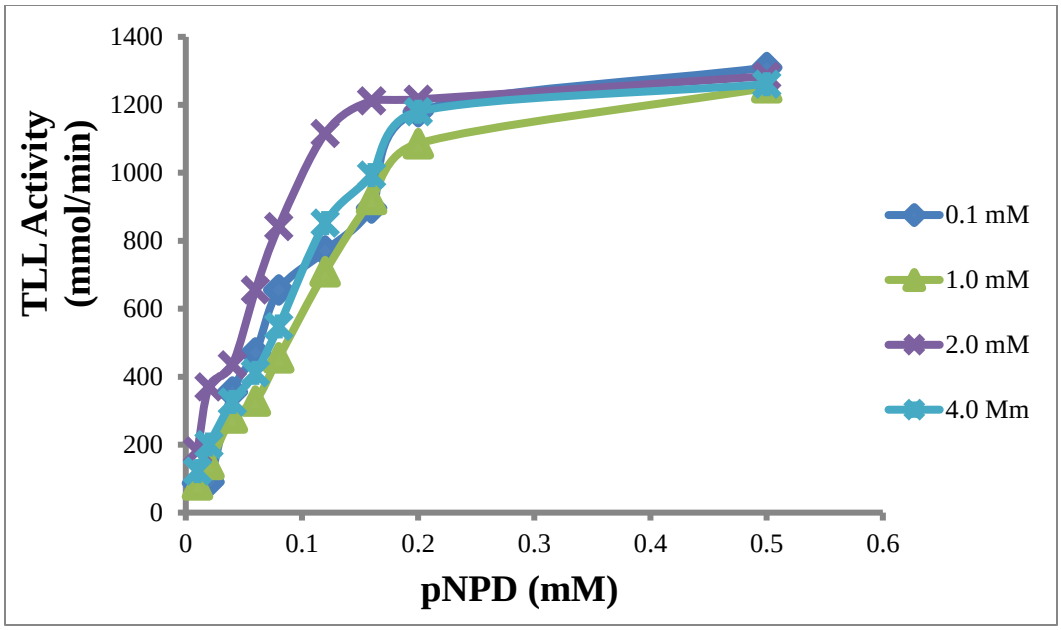

Figure 10: Michealis Menten curve of *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of urea. Data are the means of three independent assays.

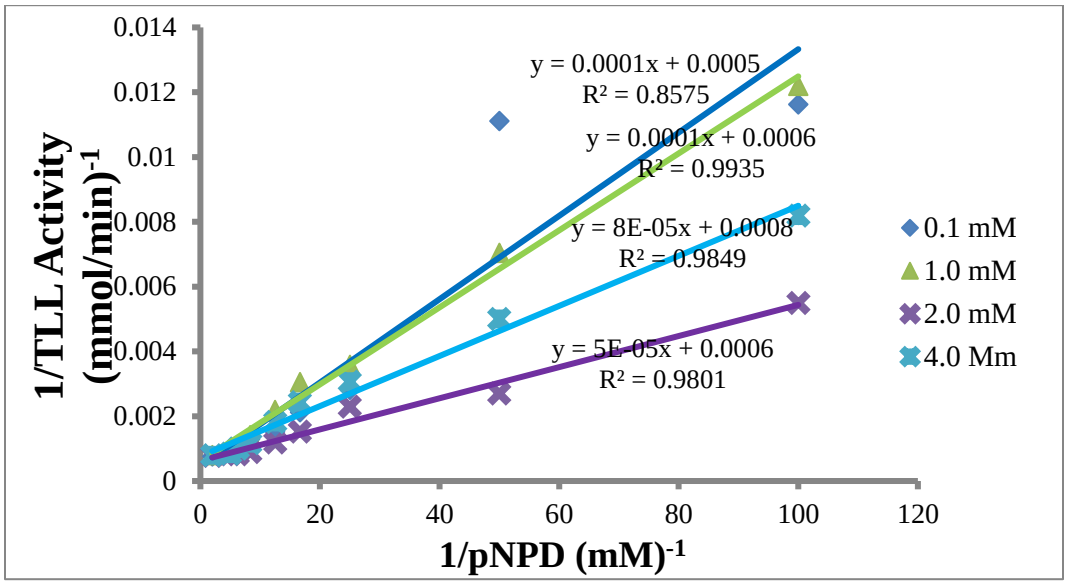

Figure 11: Lineweaver-Burk plot of *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of urea. Data are the means of three independent assays. R^2 values close to 1.0 indicates the goodness-of-fit and reliability of the data.

Kinetics of *Thermomyces lanuginosus* Lipase Catalysed Hydrolysis of para-Nitrophenyl Dodecanoate in the Presence of Sodium Dodecylsulphate

The substrate kinetics of TLL catalyzed hydrolysis of pNPD in the presence of sodium dodecyl sulfate follows the classical Michealis-Menten hyperbola curve (Figure 12). From the Lineweaver-Burk plot in Figure 13, Vmax, Km, and Kcat values were calculated as shown in Table 1. SDS at 0.1 mM decreased Vmax, Kcat and Km, by 87, 87 and 93%, respectively. 1.0 mM SDS did not affect Vmax and Kcat of TLL, but reduced Km by 50%. At 2.0 mM SDS, Vmax and Kcat increased by 43% while Km reduced by 28%. However, 4.0 mM SDS reduced Vmax, Kcat and Km of TLL by 75%.

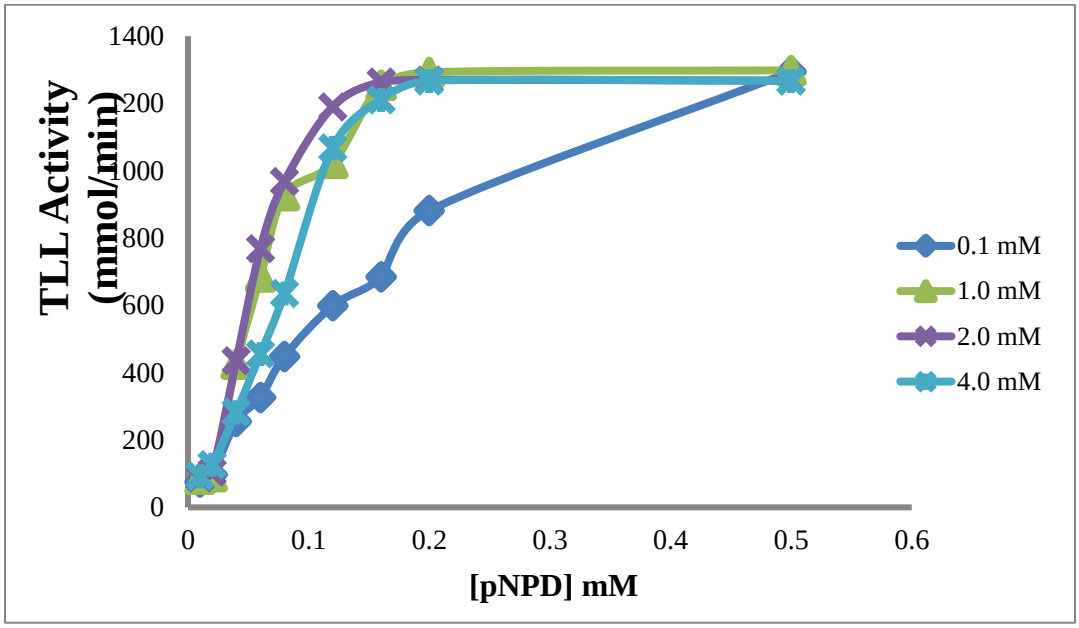

Figure 12: Michealis Menten curve of *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of sodium dodecyl sulphate. Data are the means of three independent assays.

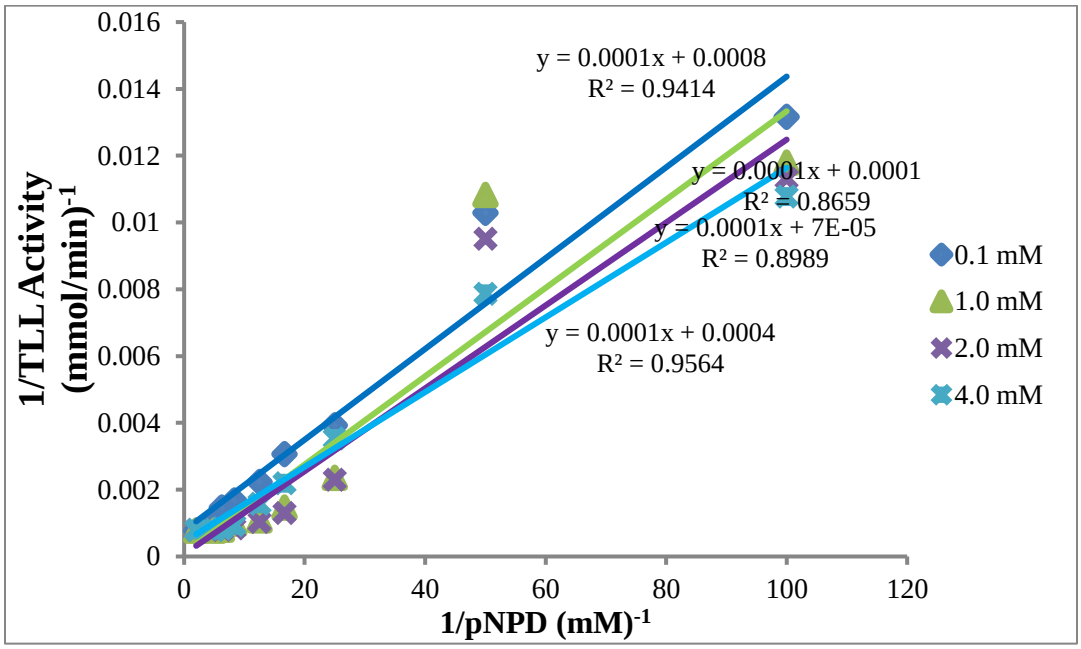

Figure 13: Lineweaver-Burk plot of *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of sodium dodecyl sulphate. Data are the means of three independent assays. R^2 values close to 1.0 indicates the goodness-of-fit and reliability of data.

Table 1: Effects of urea and sodium dodecyl sulphate (SDS) on the kinetic parameters of *Thermomyces lanuginosus* lipase catalysed hydrolysis of para-nitrophenyl dodecanoate

Reaction	V_{max} (mmol/min)$^{-1}$	K_m (mM)	K_{cat} (sec^{-1}) x 10^4
TLL	10,000.00	2.00	18.9
TLL + 0.1 mM Urea	2,000.00	0.20	3.7
TLL + 1.0 mM Urea	1,666.67	0.17	3.2
TLL + 2.0 mM Urea	1,666.67	0.08	3.2
TLL + 4.0 mM Urea	1,250.00	0.10	2.4
TLL + 0.1 mM SDS	1,250.00	0.13	2.4
TLL + 1.0 mM SDS	10,000.00	1.00	18.9
TLL + 2.0 mM SDS	14,285.71	1.43	27.1
TLL + 4.0 mM SDS	2,500.00	0.25	4.7

Discussion

Generally, lipases from microbial origin have neutral or alkaline pH optima but still display relative activity at acidic pH (Khan *et al*., 2017). This quality has made lipases more suitable (over other enzymes) for applications in industrial settings where enzymes that are effective in a broader pH range are desired (Chapman *et al*., 2018). It was observed in this study that Thermomyces lanuginosus lipase (TLL) had optimal activity at pH 9.0 but still have high and stable activity at a wide range of acidic, neutral and alkaline pH values (Figure 3). This result was in agreement with what was reported for TLL by Rodrigues *et al*. (2009), where optimal pH of TLL was 9.0. The effect of pH on the hydrolysis of olive oil by Bacillus acidophilus lipase was studied and the enzyme was reported to maintain a high activity within pH 10.0 – 11.0 (Guncheva and Zhiryakova, 2011).

One reoccurring barrier in the use of enzymes in industries where biocatalyst can be used is the lack of enzymes that are stable at high temperatures (Cao *et al*., 2016; Grigoras, 2017). Thermostability is a desirable characteristic of lipases and it has been reported that lipases have optimal activity at elevated temperatures (Khan *et al*., 2017). A previous study has shown that Thermomyces lanuginosus lipase maintains stability at 55 – 60 °C (Fernandez-Lafuente, 2010). Findings in this study demonstrate that TLL maintains relative stability at 55 - 65 °C (Figure 4). From Figure 5, it is observed that pre-incubating TLL at high temperatures yielded peak activity within the range of 50 – 65 °C. This optimal temperature range is close to Thermomyces lanuginosus optimal growth temperature of 50 – 60 °C (Maheshwari *et al*., 2016). Guncheva and Zhiryakova (2011) reported the optimal temperature of Bacillus acidophilus lipase at 60 °C. Two lipases from Thermosyntropha lipolytic (LipA and LipB) have been reported to yield broad activity maximum within the range of 86 °C to 96 °C (Salameh and Wiegel, 2010).

Small organic molecules such as urea in aqueous solutions have been found to have a significant effect on the structure, stability, and function of proteins (Canchi and Garcia, 2013). Urea has been widely used over time to assess protein stability; its characteristic denaturing effect has been attributed to its ability to cause unfolding of polypeptides (Canchi and Garcia, 2013). Urea affects enzyme structure either by direct interaction with the macromolecule or by an indirect action through effects on the structure and properties of the surrounding solvent or by a combination of both (Canchi and Garcia, 2013). Manufacturing industries of many important chemicals such as fertilizers, detergent, glue and feed supplement make use of urea (Meessen and Petersen, 2010). For TLL to be useful in such industries there is a need for it to have some degree of stability in the presence of urea. The findings from this study showed that all concentrations of Urea 0.1 to 4.0 mM increased the activity of TLL (Figure 6). This is an interesting finding since it is expected that exposing TLL especially to a high concentration of Urea (4.0 mM) would result in loss of activity due to excessive protein unfolding (Canchi and Garcia, 2013). This observation suggests that TLL resists denaturation by urea. Abuin *et al*. (2005) reported that lipase from Rhizopus arrhizus had less resistance to denaturation by urea at 2.0 M in aqueous solution. Partial

structure unfolding by urea has been reported to have reactivating potentials on TLL especially after partial inactivation by heat or interaction with organic co-solvents (Rodriguez-Larrea *et al*., 2008, Fernandez-Lafuente, 2010).

Furthermore, the kinetic analysis showed that at a low concentration of urea (0.1 mM), the turnover rate of TLL was drastically reduced (Table 1) implying that the polypeptide unfolding at this concentration had a severe impact on the maximum reaction rate (Vmax) and catalytic constant (Kcat). The concentration-dependent effect of urea on the Km of TLL suggests that the stabilized unfolded state of TLL promotes substrate binding, but did not amount to an equivalent increase in product turnover. This finding was in contrast to earlier reports where urea was observed to increase Kcat and Km of Rhizopus arrhizus lipase (Abuin *et al*., 2005).

Generally, an ionic detergent such as SDS unfolds proteins by binding unspecifically to their surface, in the case of enzymes exposing their active site to substrates (Wang *et al*., 2013). If TLL would be useful in the detergent industry as a facilitator of fat stain removal in washing powders, there is a need for it to be effective in complex mixtures of compounds including anionic and nonionic detergents (Fernandez-Lafuente, 2010). These detergents may have two effects on TLL; firstly, they are able to break the enzyme-enzyme interactions yielding momomeric forms of the enzyme that has been reported to be more active but less stable (Fernandez-Lafuente, 2010). Secondly, detergents can stabilize the open form of the lipase (Fernandez-Lafuente, 2010). From this study, SDS at 0.5 – 4.0 mM had a concentration-dependent activating effect on TLL activity (Figure 7). The findings herein showed that TLL strongly resists denaturation by SDS even at high concentrations (4.0 mM) and may be applicable in industries where moderate concentrations of a complex mixture of compounds (such as anionic detergents) are necessary for manufacturing. The increase in TLL activity can be attributed to the unfolding effect of SDS, resulting in the quicker displacement of the polypeptide lid covering the active site (Wang *et al*., 2013). Another possible reason for the increase in TLL activity in the presence of SDS is the fact that detergents, in general, are similar to the natural substrates of lipases, in that they are capable of forming higher-order aggregates/micelles (Salameh and Wiegel, 2010). The effect of SDS on alkali thermophilic lipase LipA from Thermosyntropha lipolytic has been studied and its activity was reported to increase with an increase in SDS concentration from 1.0 mM to 7.0 mM (Salameh and Wiegel, 2010).

Mogensen *et al*. (2005) identified several rapid relaxation phases when TLL was mixed with SDS between 2.0 and 5.0 mM and posited these phases to be the onset of inactivation. The findings here showed that in the presence of excess pNPD, an onset of inactivation of TLL was noticeable at 4.0 mM SDS. In contrast to the report of Mogensen *et al*. (2005), this research recorded a 43% increase in the Vmax and Kcat of TLL at 2.0 mM SDS (Table 1). This increase may be attributed to the stabilized open form state due to conformational changes induced by the detergent. At 0.1 and 4.0 mM SDS, it was observed that although Km values of TLL were low (signifying increased substrate affinity), it did not equate to increased turnover of product. This result shows that increased substrate binding to TLL has no direct connection with increased Vmax and Km. Previous studies have shown that 0.2% SDS increased Vmax of alkali thermophilic lipases LipA and LipB from Thermosyntropha lipolytic (Salameh and Wiegel, 2010).

Conclusion

In conclusion, findings from this study suggest that Thermomyces lanuginosus lipase partially and strongly resists denaturation by optimal concentrations of urea and SDS, respectively. Therefore, Thermomyces lanuginosus lipase can be optimized for industrial applications because of its remarkable thermostable property and resistance to protein denaturation.

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