Solvent-free Liquid Lipases as Potential Biocatalysts for the Degradation of Poly Lactic Acid (PLA) in Ionic Liquids

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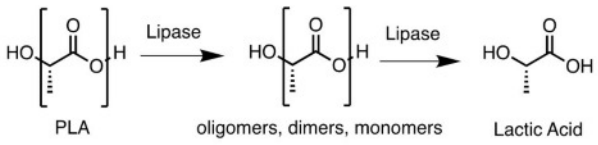
Introduction

- Poly Lactic Acid (PLA) is a polymer widely used in single use plastics.

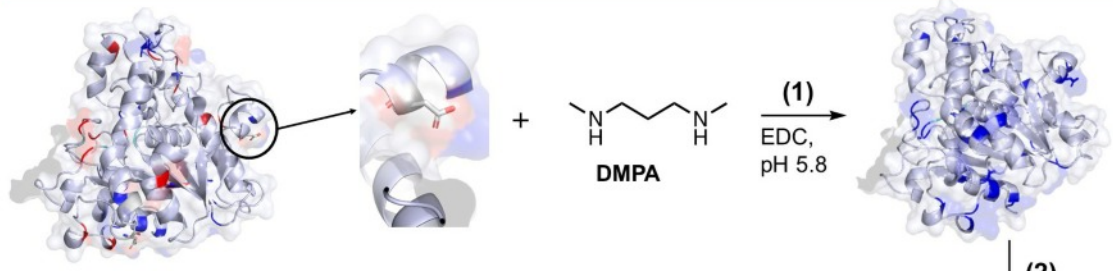


- There is now need to develop new methods to degrade and recycle PLA.
- Solvent-free liquid enzymes constructed via chemical modifications could have great potential for PLA degradation, as they have high thermal stabilities, improved enzyme activity, and high solubility in anhydrous solvents.

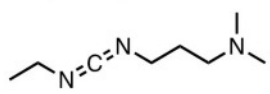
Aims

- Investigate solvent-free liquid lipase for PLA degradation
- Study how lipases' properties are affected by chemical modifications
- Optimise solubility and enzymatic activity of lipases in ionic liquids.

Protein Modification Method

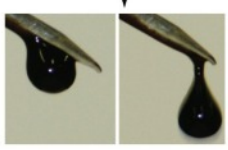


1) Cationization of surface carboxylic acid residues (Asp and Glu) using DMPA and EDC.

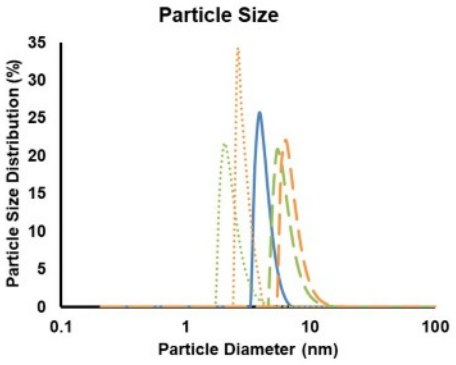


2) Electrostatic coupling of polymer surfactant.

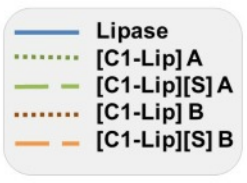
3) Lyophilization of nano-conjugated protein, followed by annealing to yield solvent-free biofluid.

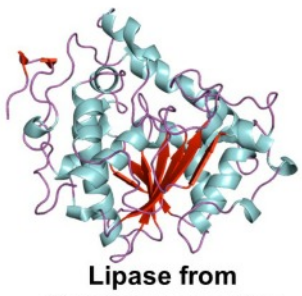


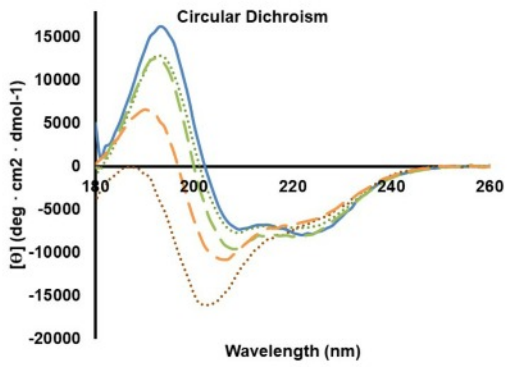
Enzyme Structure Analysis

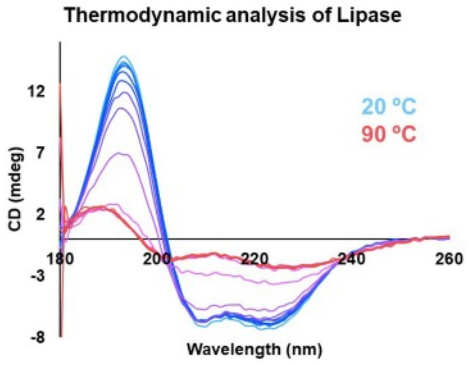


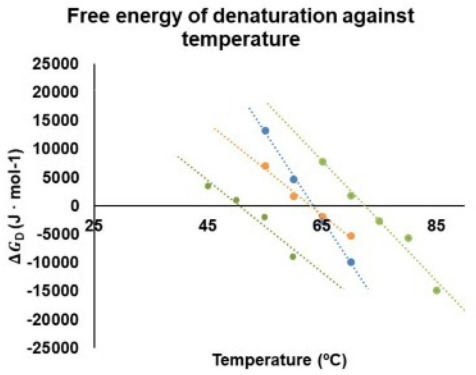
	Amines reacted	Mass (KDa)	Z-potential (mV)
Lipase	0	34.95	-1.50 ± 0.47
[C1-Lip] A	4-5	35.40	11.00 ± 0.45
[C1-Lip][S] A	-	-	-4.83 ± 0.95
[C1-Lip] B	7-8	35.72	33.88 ± 1.69
[C1-Lip][S] B	-	-	5.73 ± 0.38

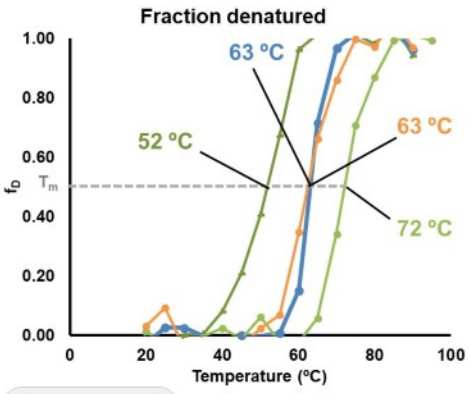










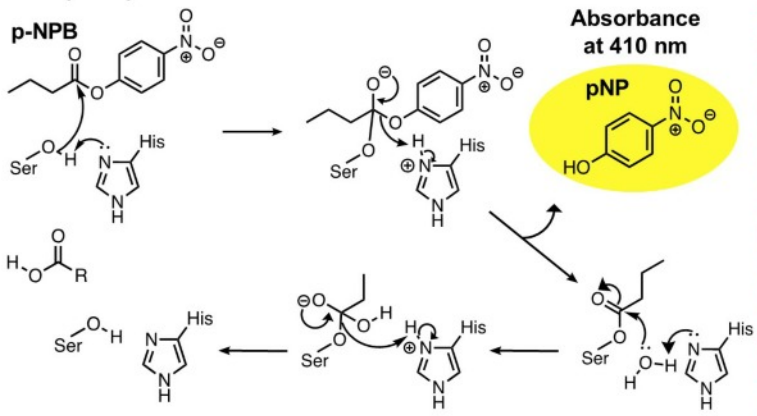


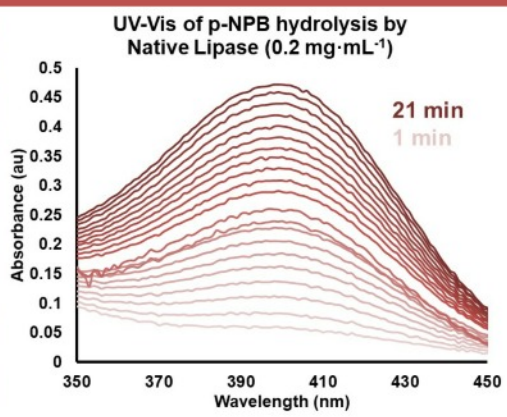
	ΔH (kJ·mol⁻¹)	ΔS (J·K⁻¹·mol⁻¹)
Lipase	970.55 ± 2.88	1529.90 ± 45.82
[C1-Lip] A	40.75 ± 7.91	806.81± 149.85
[C1-Lip][S] A	76.32 ± 8.08	1052.88 ± 107.19
[C1-Lip] B	-	-
[C1-Lip][S] B	50.77 ± 4.22	805.37 ± 67.29

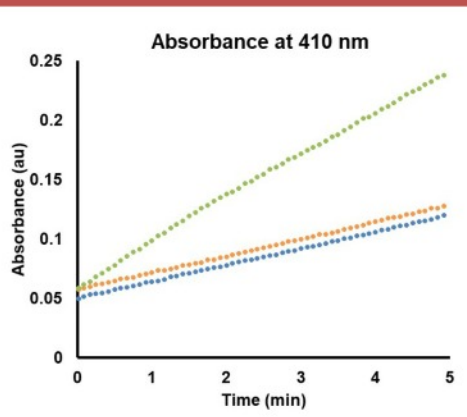
- Cationization of lipase is confirmed by ESI-MS and Z-potential (DLS).
- CD shows changes in the secondary structure of lipase after chemical modifications.
- Particle Size and Z-potential confirm nano-conjugation of lipase.
- [C1-Lip] B shows no changes in structure over temperature.
- Nano-conjugation of lipases maintains or improves native lipases thermostability: higher T_m.

Enzyme Activity Assay

- UV-Vis used to measure the initial rate of p-NPB hydrolysis







	Enzymatic Activity (μmol · min⁻¹ · mg⁻¹)
Lipase	0.98 ± 0.29
[C1-Lip] A	5.78 ± 1.73
[C1-Lip] B	2.27 ± 0.46

Cationized lipases are more active than native. Changes in secondary structure, due to chemical modifications, could expose the active site to the solvent resulting in a faster rate.

Conclusion & Future Work

- Nano-conjugation of cationized lipases has been successful. These modifications do not conserve the secondary structure of lipase. However, nano-conjugation improves thermostability.
- Enzymatic activity of nano-conjugated lipases and their reversible folding ability will be analysed. Moreover, analysis of thermostability and activity will be tested in ionic liquids. Finally, the ability of modified lipases to degrade PLA plastic will be studied.

References

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