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Versatile eutectogel-based biosensor for detecting lipase inhibitors: from obesity treatment to broader drug discovery

Raúl Martínez-Baquero, María José Martínez-Tomé, Felipe Hornos, Javier Gómez, Rocío Esquembre and C. Reyes Mateo

IDiBE, Miguel Hernández University, Elche, Alicante, Spain

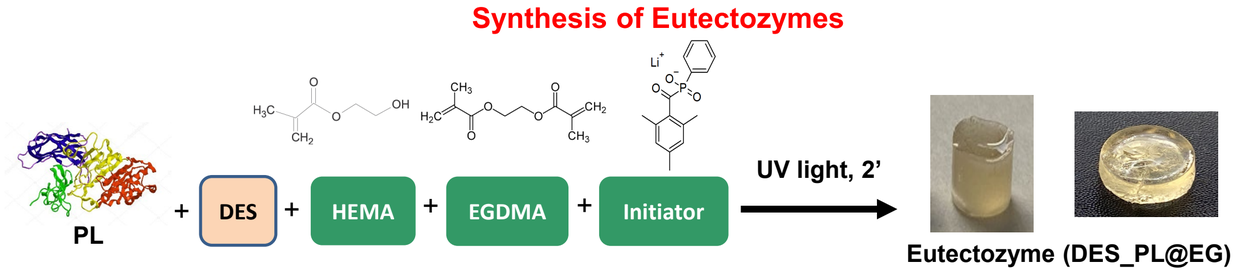
Email: raul.martinezb@umh.es

INTRODUCTION & AIM

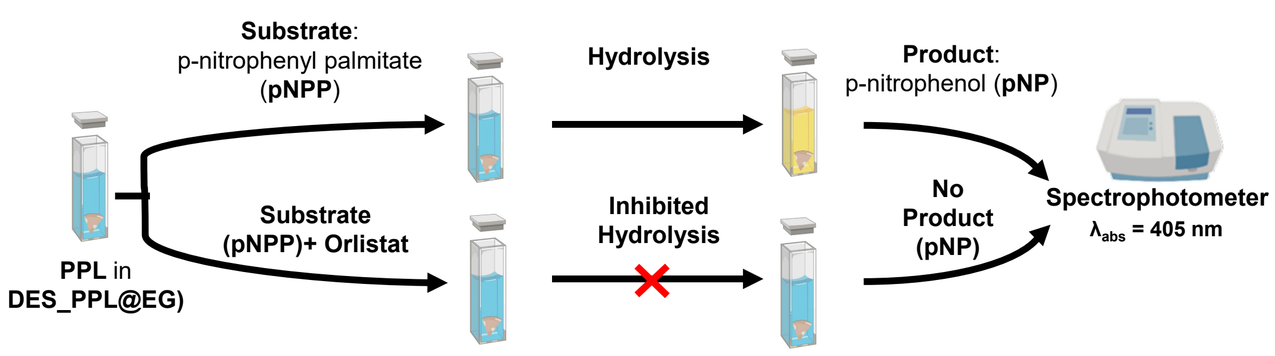
- **Deep eutectic solvents** (DES) are combinations of hydrogen bond donors (HBD) and acceptors (HBA) in specific molar ratios. They have a number of unique properties, such as low volatility, thermal stability, biodegradability and low toxicity, which provide a sustainable alternative to traditional solvents.
- When incorporated into a 3D polymeric network, DES form **eutectogels** (EGs)—hybrid materials that combine the tunable properties of DES with the mechanical stability, elasticity, and stretchability provided by the polymer matrix. Since protein immobilization enhances protein stability and reusability, the incorporation of enzymes into EGs to obtain a new material - **eutectozyme** - presents a particularly promising approach. Nevertheless, this area remains underexplored, with only a few studies reported so far.
- **Pancreatic lipase** (PL) is a key enzymatic target for screening anti-obesity compounds, as it catalyzes the hydrolysis of dietary triglycerides. In this study, this enzyme was selected for incorporation into EGs with the aim of developing a **colorimetric biosensing platform for the detection of PL inhibitors**, which may exhibit potential anti-obesity activity. Orlistat, a clinically approved PL inhibitor used in the treatment of obesity, was used as a control compound.
- EGs were prepared and characterized through UV-induced in situ radical polymerization of suitable precursors in appropriate DES. Prior to immobilization, the enzyme was solubilized in the DES formulations, and its catalytic activity and conformational stability were assessed.

METHOD

Synthesis of Eutectozymes

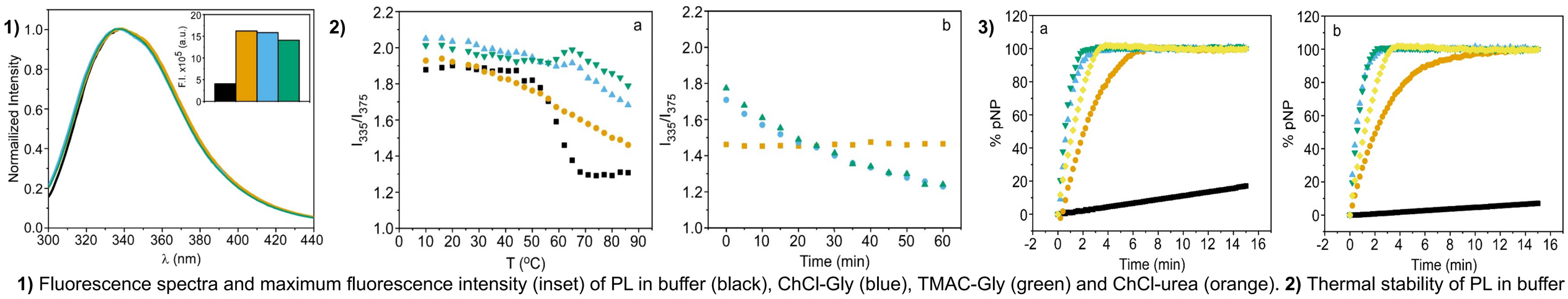


Bioensor platform operating diagram



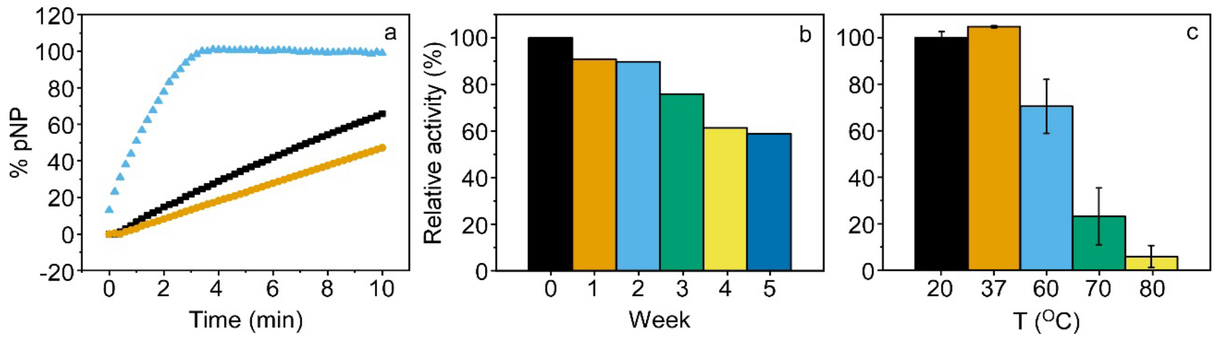
RESULTS & DISCUSSION

Characterization of PL in DES



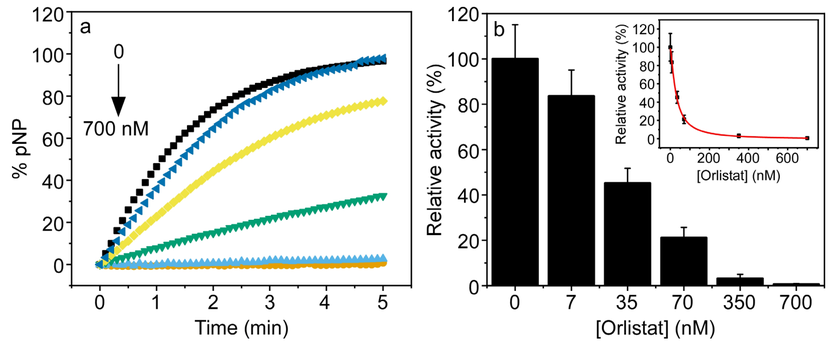
1) Fluorescence spectra and maximum fluorescence intensity (inset) of PL in buffer (black), ChCl-Gly (blue), TMAC-Gly (green) and ChCl-urea (orange). **2)** Thermal stability of PL in buffer (black), ChCl-Gly (blue), TMAC-Gly (green) and ChCl-urea (orange), monitored by changes in I_{335}/I_{375} fluorescence intensity ratio as a function of temperature (a) and its evolution during 1 hour, after temperature scan (b) **3)** PL activity, expressed as percentage of pNP formation after addition of pNPAc (Detected spectrophotometrically by recording the maximum absorption of pNP at 405 nm), measured in neat DES (black) and in DES:buffer mixtures (ChCl-Gly or TMAC-Gly) with decreasing DES content (v/v): 75% (orange), 50% (blue), 25% (green), and 0% DES (yellow).

Catalytic activity and stability of Eutectozymes



a) PL activity expressed as percentage of pNP formation after addition of pNPAc, measured in buffer (blue), TMAC_PL@EG (black) and ChCl_PL@EG (orange). **b)** Effect of storage time on the activity of TMAC_PL@EG. **c)** Thermal stability of TMAC_PPL@EG evaluated after incubation at different temperatures for 5 min and subsequent measurement of its activity.

TMAC_PPL@EG as platform for antiobesity-drug screening



a) Effect of increasing concentrations of orlistat on the catalytic actvity of TMAC_PPL@EG expressed as percentage of pNP formation after addition of pNPP. **b) and its inset** show the relative activities of TMAC_PPL@EG derived from the linear slope of the curves dispayed in a), showing the suitability of the platform to detect PL inhibitors at very low concentration.

CONCLUSION

- Dissolution of PL in neat DESs composed of TMAC-Gly or ChCl-Gly thermally stabilizes the enzyme and maintains it in a structurally stable conformation
- PL is essentially inactive in pure DES but can be efficiently reactivated upon dilution with DES concentration of 25% (v/v). These results support DESs as preservation media
- TMAC-Gly@EG and ChCl-Gly@EG behave mainly as elastic solids capable of regaining their initial form once the stress is released, without disrupting or changing their internal structure. The incorporation of PL slightly reinforces the material and increases the internal friction, but without modifying its viscoelastic behavior.
- TMAC-PL@EG activity was higher than that of ChCl-PL@EG. Its functionality was maintained for weeks, being relatively resistant to high temperatures.
- Preliminary studies show that eutectozimes have the capacity to detect inhibitors in the nanomolar range (roughly between 7 and 350 nM of orlistat). Once optimized, this system could serve as a versatile platform for drug screening against obesity and other pathologies, simply by substituting the enzyme involved.