THE EFFECT OF DEEP EUTECTIC SOLVENT AS A CO-SOLVENT ON THE HYDROLYTIC ACTIVITY OF THE METAGENOMIC LIPASE LipC12

Douglas J. S. Claudino[1], David A. Mitchell[1,2] & Nadia Krieger[1,3]*

[1]*Postgraduate Program in Science - Biochemistry, Federal University of Paraná, UFPR, Curitiba, Brazil.*
[2]*Department of Biochemistry and Molecular Biology, Federal University of Paraná, UFPR, Curitiba, Brazil.*
[3]*Departament of Chemistry, Federal University of Paraná, UFPR, Curitiba, Brazil.*
** nkrieger@ufpr.br*

ABSTRACT

With the advancement of green chemistry, the use of sustainable solvents becomes increasingly important. In particular, deep eutectic solvents (DESs) offer economic accessibility and biodegradability. These solvents have been applied in chemistry, particularly in biocatalysis, as an eco-friendly alternative to traditional organic solvents. This study evaluated the effect of DESs, as co-solvents, on the hydrolytic activity of the metagenomic lipase LipC12. The activity increased with DES concentration from 20 to 50% (v/v) capered to the reaction without the solvent, with the best performance at 20% (v/v). A DES concentration of 60% led to complete inactivation of LipC12. The results highlight the potential of DESs as co-solvents in enzymatic catalysis, provided they are used at suitable concentrations.

Keywords: Lipases. Hydrolytic activity. LipC12. Deep Eutectic Solvents.

1 INTRODUCTION

The increasing demand for sustainable chemical solutions has driven the development of environmentally friendly solvents, with particular emphasis on ionic liquids (ILs) and deep eutectic solvents (DESs).[1] Unlike ILs, DESs are less toxic and have a low production cost as they consist only of a hydrogen bond acceptor and donor (HBA and HBD).[2] They have found widespread applications in chemistry, including biocatalysis, where they stand out as alternatives to organic solvents.[3] Recent studies have demonstrated that the presence of DESs or their components can enhance the catalytic activity of enzymes, such as lipases, in reactions such as hydrolysis and esterification, highlighting the potential of these solvents as versatile tools in sustainable chemistry.[4]

Lipases (EC 3.1.1.3) catalyze the hydrolysis of triacylglycerols. They can also catalyze various other reactions in water-restricted media, including esterification and transesterification, making them important in the transformation or production of pharmaceuticals, food, and biofuels.[5] However, lipases can be deactivated by the components of the reaction medium, such as organic solvents and DESs, particularly at high concentrations. Therefore, it is crucial to evaluate the impact of DESs on lipase activity before using them in biocatalysis.

This work evaluates the impact of a DES, based on choline chloride and glycerol, as a co-solvent, on the hydrolytic activity of the metagenomic lipase LipC12.

2 MATERIAL & METHODS

LipC12 was expressed and purified from *Escherichia coli* BL21(DE3) cells transformed with plasmids containing the corresponding genes.[5] After cultivation in LB medium and induction with IPTG for 16 h at 20 °C, the cultures were centrifuged, the cells were lysed by sonication, and the crude extract was subjected to SDS-PAGE to verify protein expression. LipC12 was purified by affinity chromatography with a 5 mL HiTrap Chelating HP nickel column, followed by dialysis with a cellulose membrane, and stored at 4°C.

The deep eutectic solvent was based on choline chloride and glycerol (ChCl:Gly), in a 1:2 molar ratio. Choline chloride (ChCl) was first dried in an oven at 60 °C. Subsequently, the components of the mixture were weighed and transferred into a water bath with gentle stirring at 80 °C until a clear liquid was obtained.[6]

The olive-oil-hydrolyzing activity of LipC12 was determined in a pHStat, using different concentrations of ChCl:Gly (10% to 60%, v/v) in the reaction medium. For the hydrolysis assays, an emulsion was prepared with gum arabic, $CaCl_2 \cdot 2H_2O$, Tris-HCl, and NaCl, with the pH adjusted to 7.0.[7] Olive oil was added to the emulsion and homogenized. Then, 20 mL of the emulsion, with different concentrations of ChCl:Gly or without ChCl:Gly, was transferred to the reaction flask, thermostated at 37°C and stirred at 300 rpm. The reaction was initiated by adding 20 µL of a LipC12 solution containing 5 µg of protein, and the reaction was followed for 5 min. Initial velocities were determined. One unit of lipolytic activity was defined as the production of 1 µmol of fatty acids per min under the assay conditions.

3 RESULTS & DISCUSSION

At ChCl:Gly concentrations from 20 to 50%, the olive-oil-hydrolyzing activity of LipC12 was higher than that of the control, which consisted of the same reaction medium, but without ChCl:Gly (Figure 1). The best result occurred with 20% ChCl:Gly, with an activity of 2921 U mg^{-1}, which was 1.5-fold greater than that of the control (1965 U mg^{-1}). With 50% (v/v) ChCl:Gly, the LipC12 activity was only 1.1-fold greater than that of the control. On the other hand, 60% (v/v) ChCl:Gly caused a complete loss of ~~a~~ctivity.

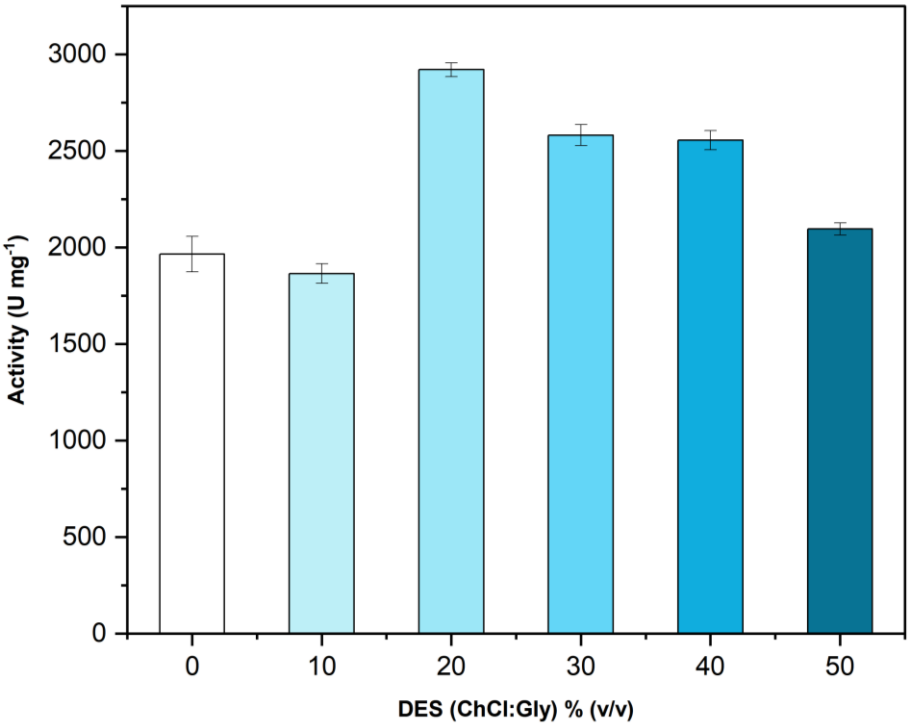

Figure 1 The effect of the deep eutectic solvent ChCl:Gly, as a co-solvent, on the olive-oil-hydrolyzing activity of LipC12.

At low DES concentrations, proteins become more flexible. Additionally, reduced DES concentrations promote the formation of a hydration layer around the active site of the enzyme, stabilizing its catalytically active conformation. As DES values increase, proteins become progressively more rigid, leading to a decrease in activity. Also, at high DES concentrations, the components of the DES can establish hydrogen bonds with the residues of amino acids in the active site, resulting in conformational changes and, consequently, a reduction in activity. [8]. Similar results have been observed in the literature for lipases from *Bacillus thermocatenulatus* (BTL2) in ChCl and urea-based DES (1:2).[9]

4 CONCLUSION

In this study, the olive-oil-hydrolyzing activity of LipC12 was affected by the presence of ChCl:Gly as a co-solvent, with the best result, a 1.5-fold increase in activity over the control, being obtained at 20% (v/v) ChCl:Gly.

REFERENCES

1 PRABHUNE, A, DEY, R. 2023. J. Mol. Liquids. 379. 2-11.
2 TANG, B., ZHANG, H., ROW, K. H. J. 2015. Sep. Sci. 38. 1055–1064.
3 PÄTZOLD, M., SIEBENHALLER, S., KARA, S., LIESE, A., SYLDATK, C., HOLTMANN, D. 2019. Trends Biotech. 37. 943–959.
4 NORO, J., CABO, J., FREITAS, D. S., ROQUE, C. S., CASTRO, M., CAVACO-PAULO, A., SILVA, C. 2023. ChemSusChem. 16. 4-8.
5 GLOGAUER, A., MARTINI, V. P., FAORO, H., COUTO, G. H., MÜLLER-SANTOS, M., MONTEIRO, R. A., MITCHELL, D. A., SOUZA, E. M., PEDROSA, F. O.; KRIEGER, N. 2011. Microb. Cell Fact. 10. 1–15.
6 SOARES, G. A., ALNOCH, R. C., ALVES DOS SANTOS, L., MAFRA, M. R., MITCHELL, D. A., KRIEGER. 2023. J. Mol. Liquids. 391. 8-10.
7 TISS, A., CARRIÈRE, F., VERGER, R. 2001. Anal. Biochem. 294. 36–43.
8 SANCHEZ-FERNANDEZ, A., EDLER, K. J., ARNOLD, T., ALBA VENERO D., JACKSON, A. J. 2017. PCCP. 19. 8667–8670.
9 SHEHATA, M., UNLU, A., SEZERMAN, U., TIMUCIN, E. 2020. J. Phys. Chem. B. 124. 8801–8810.

ACKNOWLEDGEMENTS

Scholarships were granted to Douglas Claudino by Fundação Araucária and to David Mitchell and Nadia Krieger by CNPq (National Council for Scientific and Technological Development) the Postgraduate Programs in Science - Biochemistry and Chemistry at the Federal University of Paraná. This study was funded by CAPES (Coordination for the Improvement of Higher Education Personnel) (Financial Code 001) and by CNPq (Grant nº 402085/2023-0).