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Preliminary Characterization of Cross-Linked Amidase Crystals

Hassan M. Alshamaa[1], Jesús M. Torres[3], Sergio Martínez-Rodríguez[2,3], José A. Gavira[1,2]

[1] Laboratorio de Estudios Cristalográficos. IACT-CSIC. Armilla, Granada, España. [2] Raw Materials, Human and Environmental Health, Associated Unit of the CSIC. IACT-CSIC, Armilla, Granada, España. [3] Departamento de Bioquímica y Biología Molecular III e Inmunología, Universidad de Granada, Granada, España.
email: Hassan.alshamaa.1@csic.com

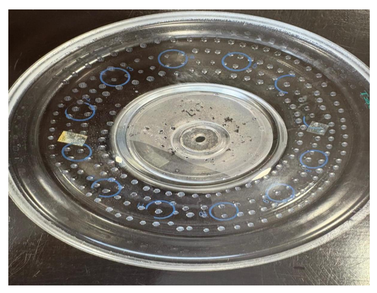
INTRODUCTION & AIM

Cross-linked enzyme crystals (CLECs) are highly purified and stabilized biocatalysts produced through enzyme crystallization followed by chemical cross-linking, which locks the enzymes into an insoluble crystalline matrix. This self-immobilization approach exhibits high stability, controllable size, and easy reuse, turning them into ideal biocatalysts. Furthermore, CLECs have been explored as microporous platforms for the controlled release of protein and peptide therapeutics, as integral components of CLECs-based biosensors, and for medical applications [2,3]. However, their principal limitation is the requirement for enzyme crystallization, an expensive and labor-intensive process that necessitates highly purified enzymes [1]. By optimizing crystal morphology and crystallization conditions, CLECs can preserve activity and selectivity similar to soluble enzymes in aqueous media and crude enzymes in organic solvents [3]. In this work, we have purified, crystallized, and produced CLECs of a new penicillin-binding protein showing D-amidase activity from *Rhizobium* species (RhiDamid). After enzyme characterization in solution, its crystallization was optimized and the resulting crystals were successfully cross-linked with glutaraldehyde to obtain Cross-Linked Amidase Crystals (CLACs), retaining D-amidase activity. Preliminary data of the characterization of CLACs is presented.

METHOD

Protein Purification

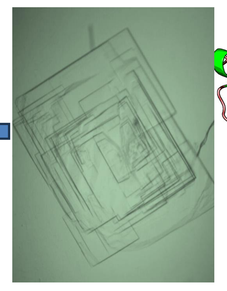
Crystallization Screening

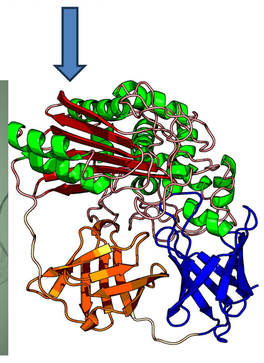


Upscaled crystallization mushroom

Washing and Centrifugation

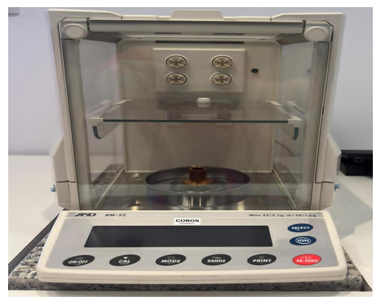
Glutaraldehyde cross-linking





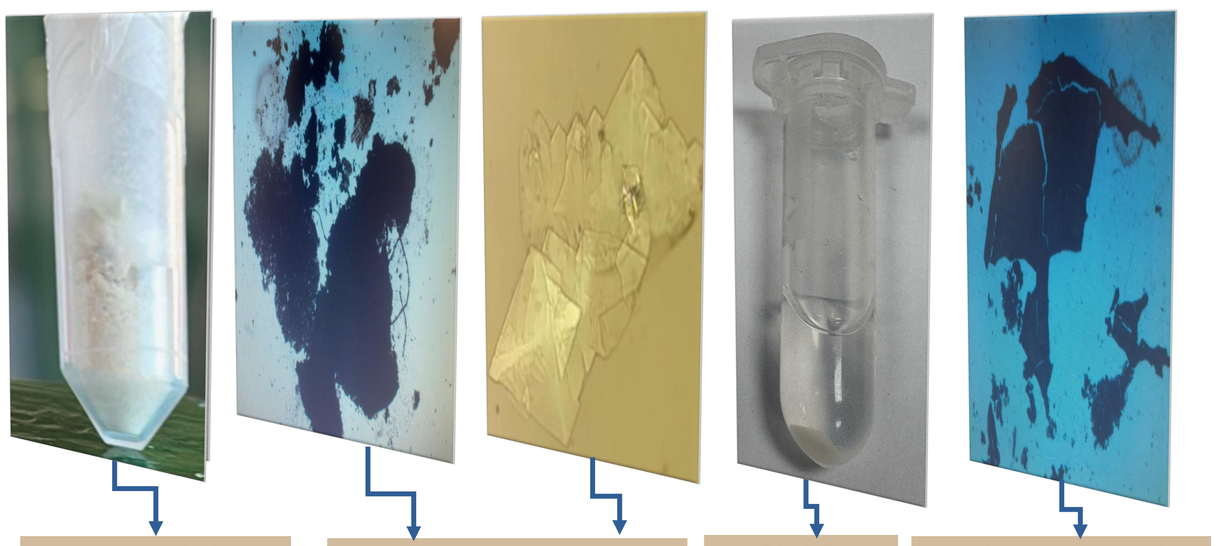
X-ray structure determination

-80 °C freezing & lyophilization



Weighing CLECs powder

RESULTS & DISCUSSION

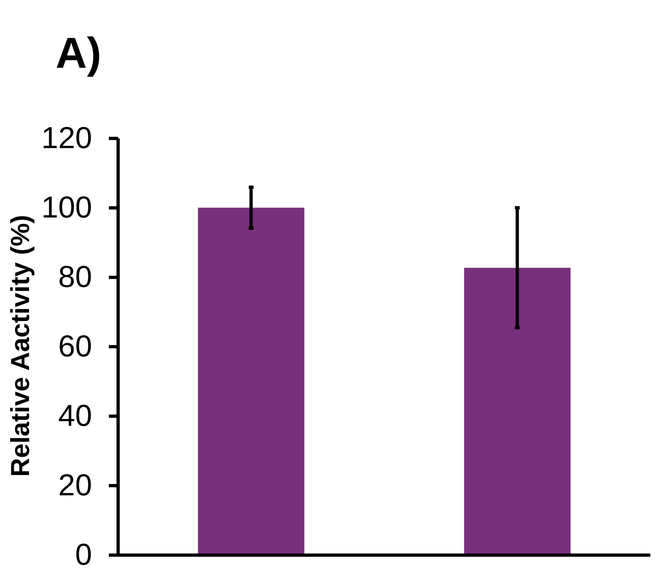


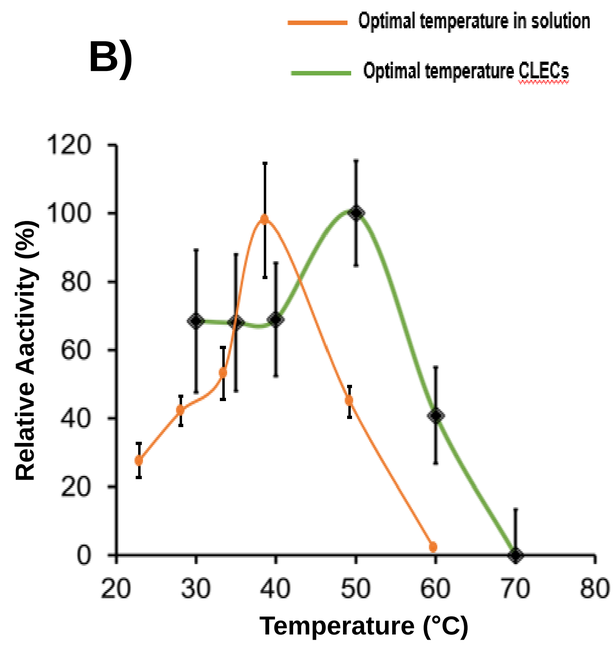
CLACs sitting-drop vapor diffusion

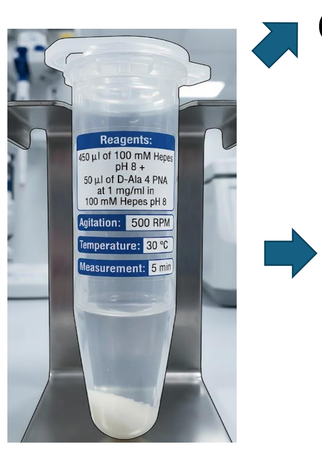
Microscopy of CLACs from sitting-drop vapor diffusion

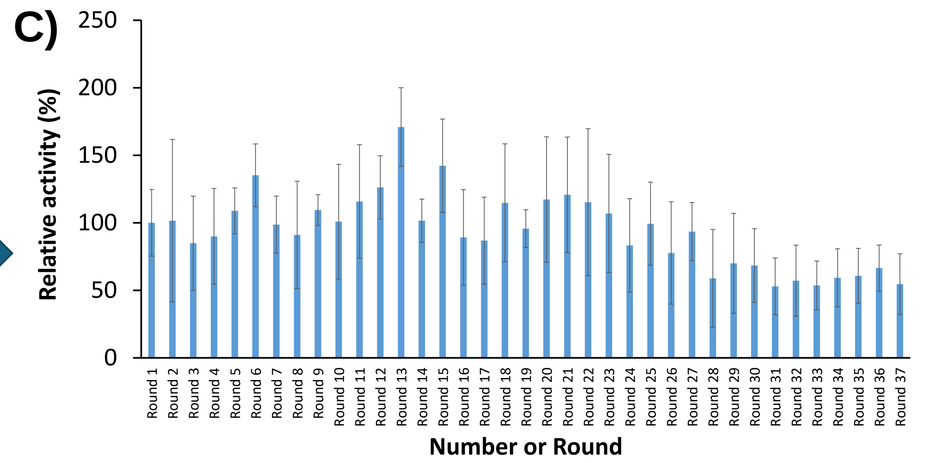
CLACs batch technique

Microscopy of CLACs from batch technique









CONCLUSIONS

A total yield of 50 mg of active CLACs was obtained using an upscaled sitting-drop vapor diffusion method. Crystals were harvested from multiple crystallization “mushrooms,” each containing up to 100 micro-drops composed of a 1:1 mixture of protein and precipitant. The batch technique also proved functional, allowing the production of active CLACs.

CLACs were initially produced from two crystallization conditions to evaluate the effect of different precipitants on cross-linking efficiency and enzymatic activity: 0,1 M Mes pH 6; 15% PEG 15-20k and 0.2 M ammonium acetate; 0.1 M trisodium citrate dihydrate (pH 5.6); 30% PEG 4K (w/v)
Both conditions were active, but for the initial experiments we proceeded with the first condition, as it was easier to handle

CLACs maintained approximately 100% of their relative activity after 1 month of storage on the bench at room temperature (Fig.A). Compared with the soluble enzyme (optimal temperature 40 °C, inactive at 60 °C), CLACs showed improved thermal stability, with an optimal temperature of 50 °C and ~40% residual activity at 60 °C (Fig.B). CLACs preserved full activity after being used more than 37 times under the same conditions and exhibited clear enzymatic activity toward D-alanine-p-nitroanilide (Fig.C).

FUTURE WORK/ REFERENCES/ACKNOWLEDGMENT

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