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Bulk Crystallization as a High-Potential Purification Strategy for C-Phycocyanin

Federica Barberio[a], Sergio Martínez Rodríguez[b,c] and José A. Gavira[a,c]

[a]Laboratory of Crystallographic Studies (IACT-CSIC), Avd. de las Palmeras, 4, 18100 Armilla, Granada, Spain. [b]Department of Biochemistry and Molecular Biology III and Immunology, University of Granada, Granada, Spain.
[c]Raw Materials, Human and Environmental Health, Associated Unit of the CSIC. IACT-CSIC, Armilla, Granada, Spain.

INTRODUCTION & AIM

Protein crystallization was originally developed as a purification strategy, exploiting its intrinsic selectivity.[1] Although chromatography later became dominant in industrial downstream processing, the increasing demand for cost-effective and scalable purification methods has renewed interest in crystallization as an alternative approach.[2]

In this work, crystallization is investigated as a preliminary purification step, requiring upstream strategies capable of increasing target concentration while limiting impurities.

Phycobiliproteins (PBPs) are used as a model system, with C-phycocyanin (C-PC, Figure 1) from *Arthrospira platensis* selected as a representative high-value target.[3]

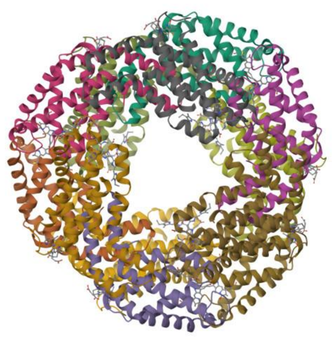
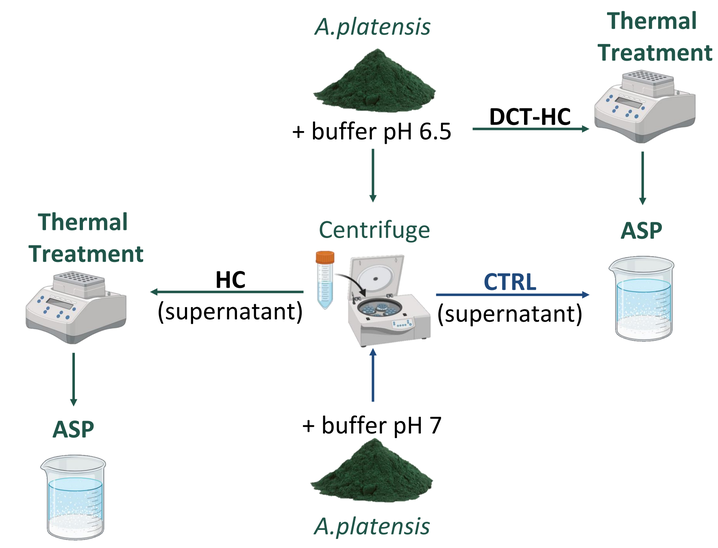


Figure 1. Crystal structure of C-Phycocyanin from *A. platensis* (PDB ID: 1GH0). (Adapted from rcsb.org).

METHOD

Three extraction strategies were evaluated: Control (CTRL), Direct Heat-Cut (DCT-HC), and Heat-Cut (HC), the latter including a thermal pre-purification step (45 °C, 10 min) before ammonium sulfate precipitation (ASP). Following ASP, samples were dialyzed, ultraconcentrated, and characterized by UV-Vis spectroscopy and SDS-PAGE.

Crystallization experiments were performed using counter-diffusion and sitting-drop vapor diffusion methods. Crystallization behaviour of crude extracts was compared with commercial C-PC.

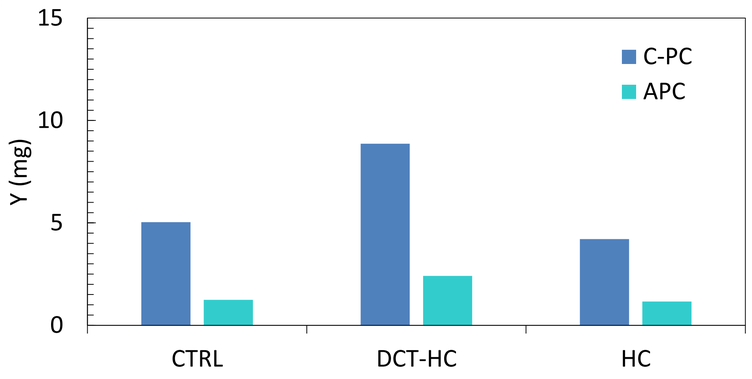


RESULTS & DISCUSSION

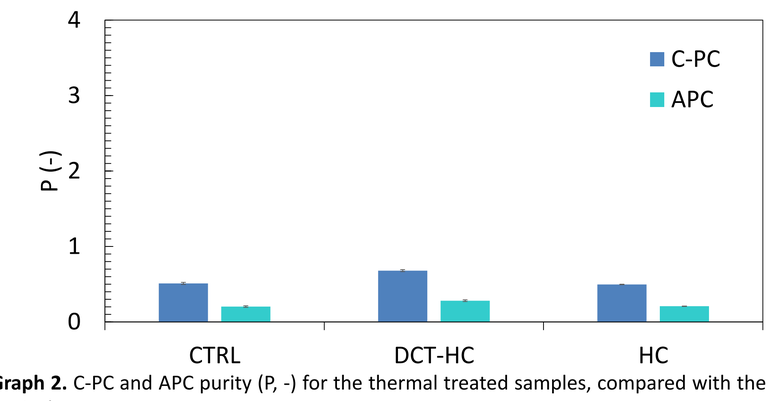
1. Recovery of PBPs

Since *A. platensis* naturally contains both C-PC and allophycocyanin (APC)[4], yield and purity indices were calculated for both proteins.

UV-Vis analysis showed the highest C-PC recovery for DCT-HC, while HC resulted in lower yields. Purity values remained low across all conditions, indicating limited purification by heat-cut treatment alone. Similar trends were observed for APC (Graphs 1-2).



Graph 1. C-PC and APC yield (Y, mg) for the thermal treated samples, compared with the control.



Graph 2. C-PC and APC purity (P, -) for the thermal treated samples, compared with the control.

SDS-PAGE analysis revealed a dominant band at approximately 18-19 kDa, corresponding to the α and β subunits of C-PC and APC. Similar electrophoretic profiles were observed among CTRL, DCT-HC and HC samples, supporting the limited purity differences detected by UV-Vis analysis (Figure 2).

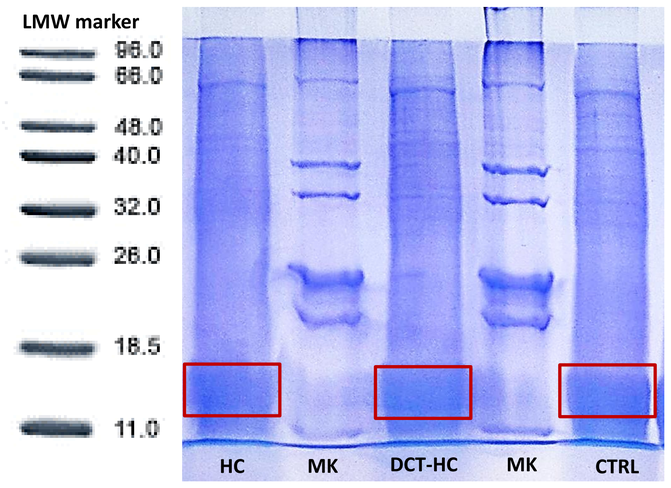


Figure 2. SDS-PAGE of thermal treated samples, compared with the control. Red boxes indicate α/β subunits (~18-19 kDa).

2. Crystallization Experiments

Counter-diffusion experiments produced small crystalline material in all three extract types. Previous diffraction analysis identified these crystals as APC, despite C-PC being the original target protein (Table 1).

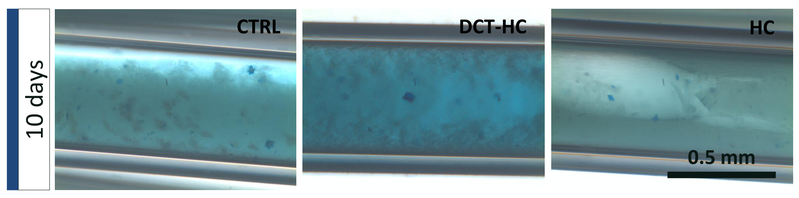


Table 1. Counter-diffusion crystallization experiments after thermal-assisted extraction. Initial APC crystal appearance after 3 days, with further development after 10 days.

This observation highlights the intrinsic selectivity of crystallization and suggests that APC may crystallize preferentially from complex *A. platensis* extracts under specific conditions.

Sitting-drop vapor diffusion screening experiments allowed to identify several crystallization conditions for commercial C-PC in common with crude algal extracts, indicating that crystallization can occur even in the presence of substantial impurities (Table 2).

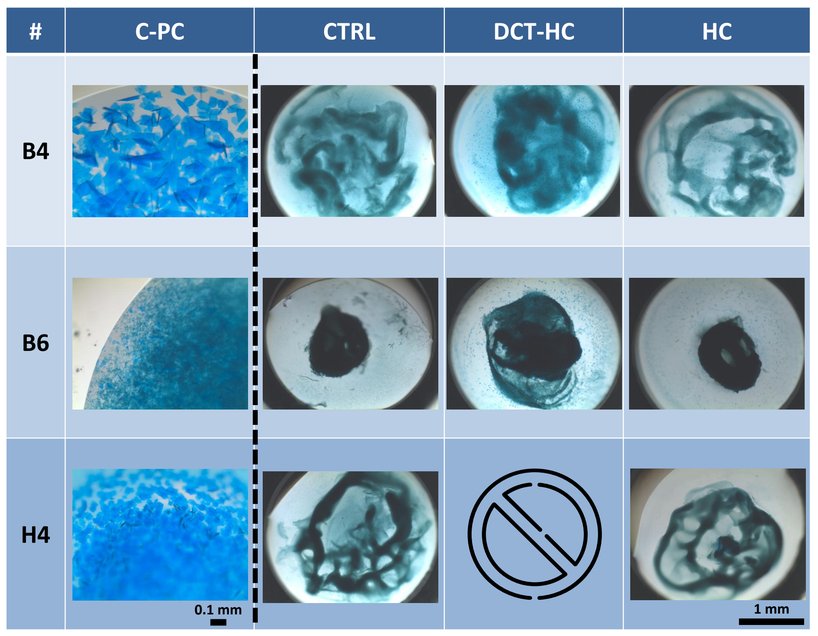


Table 2. Sitting-drop vapor diffusion crystallization experiments after thermal-assisted extraction after 1 week observation. (B4 = 20 % w/v PEG 4000; 20 % v/v 2-Propanol; in 100 mM tri-Sodium citrate, pH 5.6; B6 = 18 % w/v PEG 8000, in 100 mM MES, pH 6.5; 200 mM Calcium acetate; H4 = 30 % w/v PEG 4000; in 100 mM TRIS, pH 8.5; 200 mM Sodium acetate).

Three recurrent crystallization hits (B4, B6 and H4) were identified. B4 and B6 yielded crystals in all extracts, while H4 crystallized DCT and HC samples only. These shared hits highlight promising conditions for further crystallization optimization.

Notably, B4, B6 and H4 were also identified among the crystallization hits obtained for commercial C-PC, suggesting that these conditions may favor crystallization of PBPs even in complex algal-derived mixtures.

CONCLUSIONS & FUTURE WORK

Recurrent crystallization hits obtained under PEG 4000/8000 conditions supplemented with acetate or sulfate salts indicate promising crystallization environments for PBPs, while APC crystallization highlights the potential selectivity of the process.

Crystallization remains a promising selective approach when integrated with downstream strategies designed to enhance crystallization rather than purity alone.

Future studies will investigate crystal identity, optimize lead conditions through seeding approaches, and evaluate the feasibility of selective crystallization as a scalable purification strategy for C-PC.

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