USE OF THE STARCH BINDING CAPACITY FOR THE PURIFICATION OF RECOMBINANT PROTEINS

PAOLA AGUILERA, DANIEL GUILLÉN, SERGIO SANCHEZ, ROMINA RODRÍGUEZ SANOJA; Instituto de Investigaciones Biomédicas, Departamento de Biología Molecular y Biotecnología, UNAM; Ciudad de México, 04510; romina@biomedicas.unam.mx, paola@iibiomecias.unam.mx

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Introduction. Nowadays, purification tags are the most used tools for recombinant protein purification, usually these tags are conformed by proteins or short amino acid sequences that confer binding specificity to small ligands or another protein immobilized on a solid support (3).
Affinity resins are generally expensive and consequently not useful for large-scale protein purification. Tags such as cellulose-binding domain and the chitin binding domain, that use polysaccharides abundantly present in nature should allow the development of low cost system for recombinant protein purification (1, 2, 3). Both systems are currently used in commercial expression vectors, pET-CBD vectors (Novagen) and Impact Kit vectors (NEB).
The starch binding domain (SBD) can be found in some amylolytic enzymes, where it promotes interaction with the insoluble starch allowing hydrolysis (4). Clearly, the SBD could be used as a tag for purification or immobilization of proteins on raw starch, starch derivatives or starch analogues. Many advantages make starch an attractive, low-cost matrix for biotechnological procedures, it has natural availability and favorable chemical properties, e.g., that it is naturally particulate, inert and approved for pharmaceutical and human uses.
In order to prove the SBD as a tag for fusion protein purification, we generated two recombinant proteins for transferring the starch binding capacity to the target proteins: the fragment C of tetanus toxin and the Cys-rich protein of *Entoameba histolytica.* Proteins were purified using raw starch and the efficiency was compared to the popular histidine tag (His_{tag}).

Methods. To compare the purification capacity of each tag, dual affinity tagged version of each protein were constructed, rendering the construct 6XHis-Protein-SBD_{tag}. Products were expressed in the vector pQE31, in *Escherichia coli.* Proteins were produced intracellulary in the soluble phase and worked under native conditions. For subsequent purification, *E. coli* cells were disrupted by sonication and the collected supernatant, filtered and directly injected to a coupled β-ciclodextrin sepahrose column and a metal ion charged column (Ni-sepharose), both columns were worked on an AKTA prime system (GE Healthcare) according to manufacturer´s protocols. In the case of the purification using raw starch, the granules were directly added to the protein extract. The mixture was incubated and centrifuged to recover the starch granules. The starch pellet was washed four times and the fusion protein eluted with β-ciclodextrin (5).

Results. We observe both proteins fused to the SBD_{tag} were able to adsorb to raw starch; only the target protein remain over the starch granules after the wash process.

Figure 1 shows the purity of the Tc-SBD recovered from potato starch.

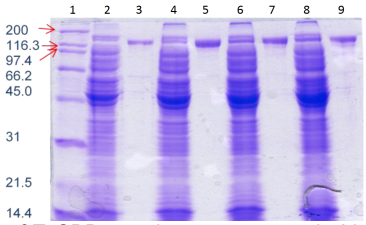

Fig.1 Purification of TcSBD protein over raw starch. Line 1, MWM broad range (Bio-RAD), lines 2, 4, 6 and 8 *E. coli* proteins, lines 3, 5, 7 and 9 purified protein after elution from the starch granules.

Figure 2 compares the purifications obtained with the His_{tag} column and the β-ciclodextrin column. In both cases, purification using the starch analogue (β-ciclodextrin) shows a better result than using His_{tag}.

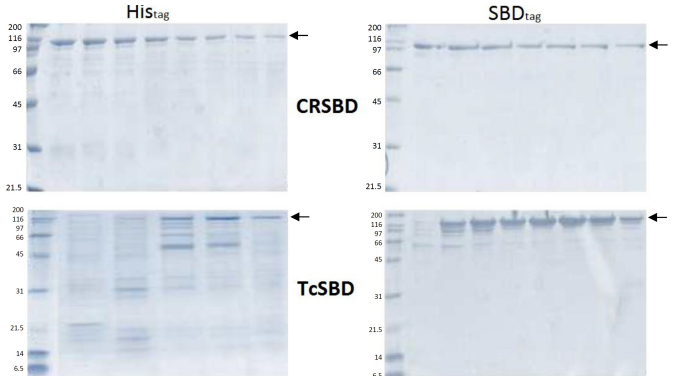

Fig.2 Purification of Cr-SBD and TcSBD. Left, purification of proteins using His_{tag}; Right, purification using the β-ciclodextrin column. On the first line MWM broad range (Bio-RAD), subsequent lines show the different fractions collected on the chromatography process.

Conclusions. Purification is possible in just one step using raw starch. Purification using the SBD_{tag} over the starch analogue was always more effective than the purification with the His_{tag} over the Ni column.
Considering protein recovery, the presence of contaminant proteins and purification cost, the SBD purification system is superior to the popular His_{tag} and useful for protein purification at any scale, laboratory or industrial.

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