iba Solutions For Life Sciences

Protein Production & Assays

STREP-TACTIN®XT VS. STREP-TACTIN® HIGH CAPACITY

Comparative analysis in a case study based on the Latex Clearing Protein

INTRODUCTION

Protein purification still is a challenging task in biochemistry. Numerous chromatographic approaches have been described ranging from time consuming purification of native proteins characterized by low yields to faster, more efficient protocols relying on tag-based affinity chromatography. Within the multitude of different tag systems, the development of the Strep-tag® brought significant improvements concerning purity, yield and ease of handling.

Latex Clearing Proteins (Lcps) are common within gram-positive microorganisms (e.g. *Streptomyces sp.* K30, Lcp_{K30}) that are able to grow on natural rubber as sole carbon source. Research interest increased recently since natural rubber is an everyday product and vast quantities of waste products thereof are permanently released into the environment. Examples range from sealings or latex gloves to tires, accounting for the largest part. The hydrocarbon poly(*cis*-1,4-isoprene) is the main component of the biopolymer and can be cleaved enzymatically by incorporation of molecular oxygen at the double bond. The reaction yields oligo-isoprenoids with terminal keto- and aldehyde groups (Fig. 1A) that can likely be used as biofuel or precursors for the synthesis of fine chemicals[1]. Thus, a comprehensive understanding of rubber oxygenases is of wide interest.

So far, only three extracellular rubber oxygenases were described and biochemically characterized[2]. Two examples, Rubber oxygenase A and B have to be purified as native proteins since multiple approaches to express and purify tagged variants were not successful[3]. Lcps, however were recombinantly expressed and successfully purified as Strep-tag® fusion proteins[4]. This application note compares the purification of Strep-tag®II-Lcp_{K30} on Strep-Tactin® high capacity and Strep-Tactin®XT demonstrating that the latter resin is the more efficient and less pricy option compared to the high capacity resin.

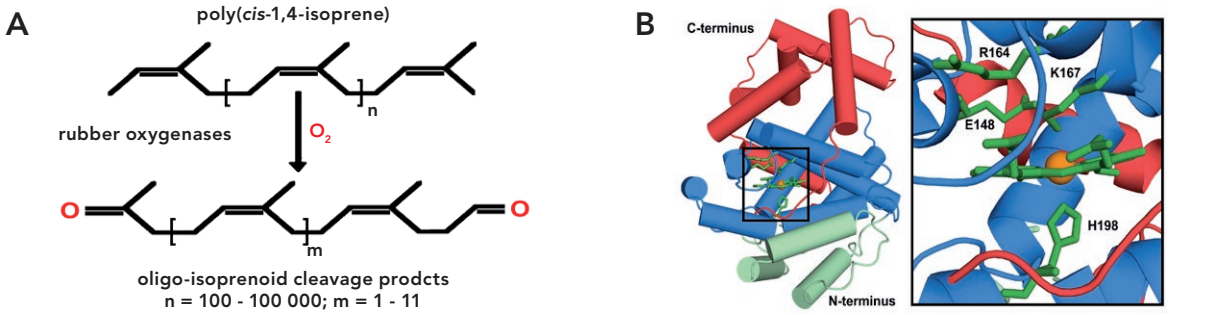

Figure 1. General properties of Lcps

(**A**) Poly(*cis*-1,4-isoprene) is the main component of natural rubber and can be cleaved enzymatically by incorporation of dioxygen at the double bond. The reaction yields oligo-isoprenoids with terminal keto- and aldehyde groups.

(**B**) The three-dimensional structure of Strep-Tag®II-Lcp_{K30} (PDB 501M) was determined by X-ray diffraction. It features a 3/3 globin fold capped by additional C- and N-terminal domains. The active site heme group and important residues H198, K167,E148 and R164 are highlighted in the inset.

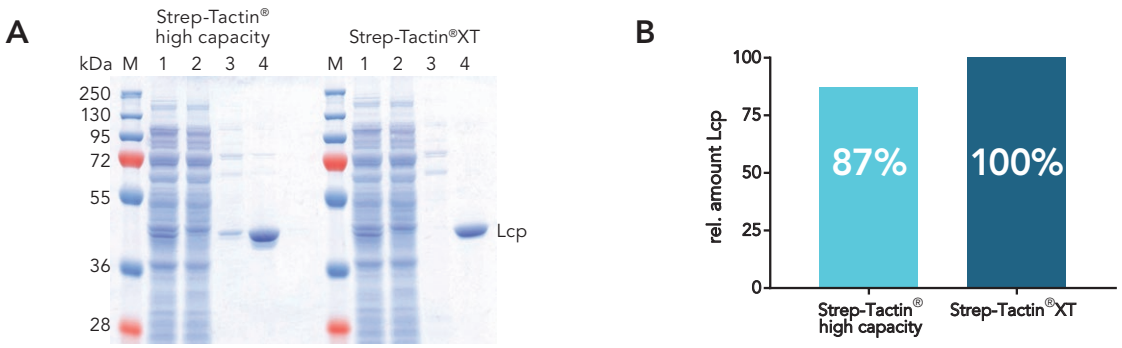

Figure 2. Comparative analysis of Strep-Tactin® high capacity and Strep-Tactin®XT
(**A**) SDS-PAGE analysis of Lcp_{K30} purified on Strep-Tactin® high capacity or Strep-Tactin®XT.
1: lysate; 2: flow through; 3: wash step no. 4; 4: elution.
(**B**) Relative amount of Strep-Tag®II-Lcp_{K30} purified on Strep-Tactin® high capacity or Strep-Tactin®XT.

RESULTS and DISCUSSION

Strep-Tag®II-Lcp_{K30} was isolated successfully to highest purity with both resins, Strep-Tactin® high capacity and Strep-Tactin®XT (Fig 2A). However, in the Strep-Tactin® high cap. purification the target protein already started to elute during the last wash step. This undesired loss of target protein was not present when Strep-Tactin®XT was used. As a consequence the protein yield differed between the two resins (Fig. 2B). It was possible to isolate ~13% more Strep-Tag®II-Lcp_{K30} with Strep-Tactin®XT than with Strep-Tactin® high cap. The higher binding affinity of Strep-Tactin®XT to Strep-Tag®II compared to Strep-Tactin® high capacity allows more intensive wash steps without the unnecessary loss of target protein. Hence, Strep-Tactin®XT enables highest protein purities under physiological conditions and sharp elution profiles for highly concentrated proteins.

Recently, the three dimensional crystal structure of Strep-Tag®II-Lcp_{K30} was determined (Fig. 1B)[5]. High binding capacities of Strep-Tactin®XT resins allow the fast purification of large amounts of pure Strep-Tag®II-Lcp_{K30} and the mild elution conditions preserve the enzyme's activity. Future improvements of Lcps by protein engineering seem possible and could further enhance their stability and catalytic turnover. This offers the future possibility to utilize Lcps for the sustainable generation of oligo-isoprenoids or energy dense biofuels derived from rubber waste[1].

MATERIAL and METHODS

Eight 3-liter Erlenmeyer flasks containing 600 ml LB medium were inoculated with approximately 0.02 volume of a seed culture of *E. coli* JM109 harbouring the plasmid p4782.1::lcp_{K30} that had been grown with the same medium. Expression of Strep-Tag®II-Lcp_{K30} was induced by addition of L-rhamnose (0.1%, wt/vol) right after inoculation. Cells were cultivated at 22° C upon shaking at 120 rpm. Cells were harvested by centrifugation (16000 *g*, 1 hour, 4° C) after 24 hours of growth and immediately used for protein purification. The cell pellet was resuspended in 100 mM potassium phosphate buffer (pH 7.8), containing 150 mM sodium chloride (KPN buffer). A soluble cell extract was prepared by two French press steps in the presence of DNAse and subsequent centrifugation (40,000 *g*, 40 min, 4 °C). 2.5 ml each of the supernatant were applied to Strep-Tactin® gravity flow columns equilibrated in KPN buffer. Subsequently, the columns were washed 4 times with KPN buffer prior to elution of Lcp_{K30} by addition of 5 mM desthiobiotin (for Strep-Tactin® high capacity) or 50 mM biotin elution buffer BXT (for Strep-Tactin®XT).

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ACKNOWLEDGEMENTS

IBA GmbH gratefully acknowledges the support of Wolf Röther, Institut für Mikrobiologie, Universität Stuttgart, Germany, during the planning and preparation of this note.

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