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Poster Presentation

Automated purification of soluble histidine tagged integrase of *Tn21* expressed in *E. coli* cells in low amounts

Ioana Grigorescu*[1], Anna Andersson[1], Markus Galin[1], Anita Jönsson[1], Anders Molin[1], Susanne Nyholm-Westin[1], Carolina Johansson[2], Lars Sundström[2] and John Flensburg[1]

Address: [1]GE Healthcare, Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden and [2]Department of Medical Biochemistry and Microbiology (IMBIM), BMC, Box 582, SE-751 23 Uppsala, Sweden

* Corresponding author

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Background

Deca-Histidine tagged integrase *Tn21*, a basic DNA-binding protein with a molecular mass of 38 kDa, when expressed in *E. coli* bacteria resulted in inclusion bodies. To obtain native, biologically active protein, it was decided to purify only cytoplasmic soluble integrase, present in low amounts. The optimal growth conditions, low temperature and 20 hours of growth period were used to cultivate the bacterial cells to increase the amount of the soluble integrase. LC-MS/MS techniques were used as analytical techniques to measure concentration of the expressed protein during growth optimization.

Results

ÄKTAxpress™, an automated system, which allows multi-step purification of protein, was used to purify the enzyme. Additional ready-to-run protocols increased the flexibility of combining different chromatographic techniques (affinity and ion exchange chromatography, gel filtration and desalting) in the desired order.

All purifications were performed at + 6 °C as the protein is labile. The method consisted of an immobilized metal chelating chromatography as capture step. A column prepacked with Ni Sepharose™ High Performance was used to capture the deca-histidine tagged integrase. The system allowed the automated collection of peaks in the storage loops and the transfer of the major peak to a gel filtration column (Hi Load™ 16/60 Superdex™ 75 prep grade). The purified protein was analyzed using SDS-PAGE and MS techniques.

Conclusion

Using the optimized experimental conditions, 5 mg of pure labile integron integrase of *Tn21* was purified, sufficient enough for functional studies as well as crystallization screening.