Study on the efficient purification of recombinant proteins from the silkworm expression system

（課程博士・様式７）（Doctoral qualification by Coursework, Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

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Abstract : Many expression systems have been used for the expression of recombinant proteins. Well-known and widely used system is *E. coli*, but it is not able to undertake post-translational modifications and can have problems with protein solubility for eukaryotic proteins. In opposite, yeast strains, don't form inclusion bodies and can modify proteins with post-translational modifications. However, production capacity can be lower, the assembly of multimers cannot be always supported, proper folding can be impossible and glycosylation patterns are not suited. Insect cell-based systems are another option, because they provide post-translational modifications, have a high growth rate and can be used for large-scale production. However, infection of a constructed vector from the recombinant baculovirus containing the gene of interest is necessary. Although the systems of *E. coli* and yeast are still the preferred platform for most protein expressions, but mammalian proteins often require eukaryotic cells for optimal yields and activity of the desired recombinant protein. Mammalian cells are more complex to construct and handling, but they are fully capable of post-translational modification and of assembling multimers. In opposite, the Bac-to-Bac system for insect cells, e.g. silkworm (*Bombyx mori*), is comparably cheaper and can provide the same posttranslational modifications as insect cells and has for some proteins even higher protein yields than the aforementioned systems. Depending on the recombinant protein, they will be released into the hemolymph or remain inside the fat body. Asian countries already possess the necessary infrastructure for rearing silkworms and the use of silkworms is also comparatively cheap, and they require only a simple, artificial diet. Moreover, only approximately 1–2 weeks are enough for the entire process from infectious vector preparation to protein harvesting. However,

purification from silkworm compounds is not an easy undertaking because of the high amount of proteins. Moreover, only few reports exist for general protein purification from the silkworm, and only some attempted a purification optimization or an upscaling. Usually non-scalable processes such as protein tags or sucrose gradient centrifugation were used. This doctoral study focused on the investigation, exploration and optimization of the protein purification from silkworm larvae for industrial use.

During this work the first attempts of purification optimization from silkworm larvae were successfully performed. For internal use, data about host cell protein behavior during each purification method was acquired. Proteins such as modified GFPuv-ß1,3-N-acetylglucosaminyltransferase 2 (GFP-ß3GnT2), mCherry, non-enveloped norovirus-like particles (NoV-LPs) and human papillomavirus-like particles (HPV-LPs), or *Rous sarcoma* virus-like particles were used for purification. Based on pre-treatment and chromatography investigations with GFP-ß3GnT2 an up-scalable purification protocol from silkworm hemolymph was established using mCherry as model fluorescence protein. Centrifugation (17800 × g, 10 min), 2.5% polyethylene glycol precipitation (PEG) and a thermal treatment with 70°C for 20 minutes were used as the pre-treatment. This was followed by a one-step gradient hydrophobic interaction chromatography (HIC) with a butyl column and 3 mol/l NaCl in binding buffer, size exclusion chromatography (SEC) and heparin chromatography and resulted in a recovery of 5.78 % with a purity of 43.60 % in regards to capillary electrophoresis (CE), whereby the purity with the Strep-tag affinity chromatography was 63.69 % and the recovery only 4.09 %. The biggest impact had hereby the thermal treatment, but this limits the protocol only to partial thermal stable proteins. However, the introduced thermal treatment result made the PEG precipitation and the SEC unnecessary, compared to a protocol without thermal treatment. Furthermore, HPV-LP purification from the silkworm fat body using anion exchange, ceramic hydroxyapatite and heparin chromatography steps was successfully achieved, reported and resulted in over 99.3 % removal of DNA and proteins. The purity exceeded that of the sucrose gradient centrifugation and even that of Flag-tag affinity purification. Another ongoing project is the pre-treatment improvement with nickel-conjugated magnetic nanoparticles (MNPs), using multiple His-tagged recombinant proteins. So far, highly dispersible MNPs were selected from 4 kinds of MNPs, their binding to several His-tagged proteins was confirmed and an up-scaled pre-treatment of NoV-LPs was successful from the complex sample matrix of the silkworm fat body. Moreover, via transmission electron microscopy the binding ability against NoV-LPs was confirmed.