Purity of maltose-binding protein - Recombinant streptavidin expressed in *Escherichia coli* BL21 (pD861-MBP: 327892)

**Abstract**

Nearly 95% of streptavidin which is expressed in *Escherichia coli* found as an inclusion body. Protein expressed in an inclusion body form requires further steps for the folding process related to its purification. Whereas the purity level of the recombinant streptavidin is very crucial mainly for the specification test in diagnostic system. In this study, we designed synthetic gene of streptavidin to be fused with maltose-binding protein (MBP) gene to enhance its solubility when expressed in *E. coli* BL21 (pD861-MBP: 327892) and purified using amylose resin with gradient column buffer. Based on the SDS-PAGE characterization, the majority of recombinant streptavidin was found in soluble than that of insoluble form. Recombinant streptavidin was found at its suitable size at 56.6 kDa in the soluble protein fraction with a concentration of 537.42 mg/L. The purest fraction of streptavidin recombinant was obtained at the 58th fraction in a concentration of 0.86 mg/L with purity level of 98.77%. Compared to the initial crude protein extract, the level of purity is lower, 6.03%. In summary, the MBP purification method improves the purity level and enhances the solubility of the recombinant streptavidin.

**Key words:** *Escherichia coli* BL21 (pD861-MBP: 327892), maltose-binding protein, recombinant, soluble, streptavidin

**INTRODUCTION**

Immunochromatography generally uses immunoglobulin G antibodies as analyte detection because it can bind specifically to antigens and can be strongly adsorbed on nitrocellulose membranes. This attachment is carried out by direct physical adsorption which allows the orientation of antibodies at random so that the active site of antibody binding can be blocked.[1,2] Therefore, it is necessary to develop an antigen-binding agent that can immobilize target proteins on nitrocellulose membranes with proper orientation and high homogeneity. The streptavidin-biotin system can be chosen as a strong candidate as a protein immobilization solution on nitrocellulose membranes because it has several advantages such as, until now the bond between streptavidin-biotin is the strongest noncovalent bond (Kd ~10–14), resistant to organic solvents, detergents, proteolytic enzymes, and extreme changes in temperature and pH.[3,4] Streptavidin can be conjugated with other proteins or tagged with various...

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Maltose-binding protein (MBP) is used as a fusion protein in recombinant streptavidin expression because of its several advantages over other fusion proteins which can support the purification process with only one stage using amylose resin and gives higher yields. MBP can be chosen as a fusion protein that improves the protein solubility and is used to prevent the formation of inclusion bodies, especially in recombinant proteins, that have low solubility when expressed on E. coli. Recombinant proteins that have not completely folded will undergo several binding processes and release from MBP. MBP can facilitate interactions between recombinant proteins that have not yet been completely folded with one or more endogenous chaperons.

MATERIALS AND METHODS

Materials

The materials used are Luria-Bertani agar (Merck), SDS-PAGE components, ethanol, synthetic gene pD861-MBP: 327892 (synthesized in ATUM, California, USA), L-rhamnose (Sigma-Aldrich), amylose resin (BioLabs), and other common chemicals for SDS-PAGE.

Construction of synthetic recombinant streptavidin genes

In this study, the synthetic gene encoded recombinant streptavidin was obtained from www.ncbi.nlm.nih.gov with access number KT344129.1. The amino acid sequence of this protein was composed of:

Menlytqsaegagtqtwynqlgstfivtag Adgaltqyesavgaesr Yvltqayds Apatdqsagtalgtavkwknyrm Haattwsqyvgga Earintqwlilt Gtgeanwstvghdfkvkpsaasykdiddk.

In this amino acid strand, there is an ENLYFQSS sequence which is the specific side to be cut by TEV protease enzyme.

Therefore, the recombinant streptavidin can be purified from MBP protein as the fusion tag protein. In addition, there is a DYKDDDK sequence which is FLAG marker amino acid sequence that serves to isolate recombinant streptavidin or can be used for quantification using enzyme-linked immunoassay method. Overall, the gene construction expressed containing 435 bp can be illustrated as shown in the Figure 1.

Figure 1: Recombinant streptavidin gene mapping. Notes: T = specific side to be cut by TEV protease; F = FLAG marker
Purification of streptavidin recombinant proteins

A volume of 3-mL amylose resin was added into the One Med Disposable Syringe 5 cc column then the resin was washed with 15-mL column buffer (5 CV). The resin was equilibrated with 24-mL column buffer (8 CV) and then 4.09 mL of sample was added and eluted with 36-mL column buffer (12 CV), then re-eluted with 36-mL column buffer containing 10-mM maltose (12 CV). The fraction was collected and characterized by UV–Vis spectrophotometer at a wavelength of 280 nm. The peaks of fraction were characterized by SDS-PAGE, and their protein total content was quantified using Lowry method and ImageJ analysis program. Characterization of recombinant streptavidin in SF was carried out using the SDS-PAGE method.

RESULTS AND DISCUSSION

We produced streptavidin by expressing the synthetic gene encoding streptavidin in E. coli BL21 (DE3). The synthetic gene was expressed better than that of identical native gene. In addition, the advantages when using synthetic genes are rapid process, no cell extraction needed and dependable access to obtain cDNA target sequences in gene bank. Moreover, the level of synthetic gene expression can be regulated by designing the expression vector component containing the target gene. Promoter, enhancer elements, signals, and restriction sites of expression system can be incorporated into the vector to elevate the yield of protein recombinant correctly.[13] Unfortunately, the expression sometimes leads to the formation of insoluble or misfolded proteins. However, this problem could be rectified by choosing the suitable codon usage to be expressed in the cell host.[14,15] From the graph in Figures 2 and 3 shown that the adaptive percentage of the streptavidin original codon <100% in E. coli. These differences will not be optimal to be read and expressed in E. coli.[16]

In Figure 4, the optimized codon sequence of streptavidin gene has changed to 100%, consistent with the codon preference in E. coli. Then, the optimized streptavidin gene sequence was inserted in the expression vector, shown in Figure 5. This plasmid vector consists of kanamycin-resistant antibiotic gene marker, rhaBAD promoter, strong RBS, initiator of Ori_pUC replication, and MBP as fusion protein that can increase the solubility of recombinant streptavidin. This plasmid vector can then be directly transformed into the host E. coli BL21 (DE3).

As illustrated in Figure 6, in the Petri dish A (positive control), the pure strain of E. coli BL21 (DE3) colonies was found on the growth media without kanamycin. In contrast to Petri dish B, when the pure strain was growth in the media containing kanamycin, then there was no colony found. However, the
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in the exponential phase, based on the procedure suggested by ATUM, the induction is carried out when the OD600 value is in the range of 0.6–0.8. The growth curve showed that OD600 values began to enter the range between the 2nd and 3rd h. Production curve of recombinant streptavidin expression, shown in Figure 8. As the theory, most of recombinant streptavidin was expressed in the SF, shown in Figure 9. The highest protein content in SF at 5th h in a concentration of 0.532238 mg/mL.

The expression fraction in the form of IF, SF, and M was analyzed to determine the presence of recombinant streptavidin. Recombinant streptavidin with its suitable size (56 kDa) was detected, presented in Figure 10, and the estimated recombinant streptavidin content of the expression was 537.42 mg/L. Theoretically, the recombinant protein is mostly found as the SF in E. coli due to the influence of MBP fusion protein that can increase the solubility of recombinant protein and minimize the formation of inclusion in IF.[17-20]

The streptavidin recombinant protein was purified by the affinity chromatography method, shown in Figure 11. The 3rd–15th fraction was detected in 280 nm and it was recharacterized using SDS-PAGE to confirm the purity level of the purification method, as shown in Figure 12. The analysis showed that the level of streptavidin recombinant purity was not good enough. Table 1 and Figure 13 demonstrated the purest fraction (98.77%) was at 58th fraction and the recovery was 6.80%.

Amylose resin is used as an affinity matrix to separate target proteins fused with MBP by the principle of gravity-flow column. Thus, the target protein can be isolated specifically by one purification step. In this study, the purest fraction was fraction 58 in purity level at 98.77% and its recovery is 6.80%. The purity level of the isolated protein using amylose resin can be affected by several factors, such as the weak binding due to the production of amylase which can interrupt amylase thus we added the glucose into the growth medium to restrain amylase expression.
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CONCLUSION

The MBP fusion to monomeric recombinant streptavidin expression was successful in enhancing the solubility of streptavidin protein produced by E. coli BL21 (DE3) and can be purified using amylose resin.

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Conflicts of interest
There are no conflicts of interest.

Table 1: Purification results

| Sample  | Vol (mL) | RSA content (mg/mL) | Protein total content (mg/mL) | Total RSA content (mg) | Total protein content (mg) | Specific content (mg/mL) | Purity (%) | Purity (fold) | Recovery (%) |
|---------|----------|---------------------|-------------------------------|------------------------|---------------------------|---------------------------|------------|---------------|--------------|
| CE      | 4.09     | 3.06±0.001          | 50.68±0.002                  | 12.49±0.003            | 207.13±0.002              | 0.96±0.000                | 6.03±0.000 | 1.00          | 100.00±0.000 |
| Fraction 58 | 1       | 0.85±0.000          | 0.86±0.001                   | 0.85±0.000             | 0.86±0.001                | 0.99±0.001                | 98.77±0.000 | 16.37         | 6.80±0.000   |

CE: Crude extract, RSA: Recombinant Streptavidin

Figure 7: *Escherichia coli* BL21(DE3) (pD861-MBP) growth curve

Figure 8: *Escherichia coli* BL21(DE3) (pD861-MBP) production curve

Figure 9: SDS-PAGE electropherogram of soluble fraction streptavidin recombinant (56 kDa)

Figure 10: SDS-PAGE electrophoregram of recombinant streptavidin soluble fraction

Figure 11: Graph of purification streptavidin recombination
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