Molecular cloning and genetic engineering - Purification of Target Protein in Vitro

Aim

The expression vector pET28a (+) contained in this laboratory contains a sequence encoding polyhistidine, that is, His-Tag. Therefore, a recombinant target protein with His-Tag will be obtained. His-Tag can bind to metal Ni^{2+} ions, which is beneficial to the purification of target protein. The protein added with His-Tag can be purified by Ni^{2+} affinity chromatography column under non-denaturing conditions. The principle of purified protein is that when the affinity column is loaded with Ni^{2+}, it can selectively adsorb amino acid residues (especially histidine residues) which are exposed to protein surface with complex structure. The more histidine the protein contains, the higher the specificity of its binding to Ni^{2+} will be, and the higher the concentration of imidazole will be required for its elution. The target protein with histidine label has higher specificity for Ni^{2+} binding than the total protein in cells. In order to obtain the target protein with high purity, it is necessary to find a suitable eluent (binding buffer) of imidazole concentration, at which the non-specific impurity protein can be eluted from the column, while the target protein specifically bound to Ni^{2+} will not be eluted. Finally, the target protein was eluted with a higher concentration of buffer (elution buffer), at which time the

target protein existed specifically in the elution solution of imidazole concentration, and the purified target protein was obtained.

Material

Starting buffer(phosphoric acid buffer)

Starting buffer(phosphoric acid buffer. containing imidazole)

imidazole

2XSDS Gel Sampling Buffer

Procedure

1. Ni^{2+}-NTA affinity chromatography column is prepared and eluted slowly with deionized water to avoid introducing bubbles into the column bed.

2. Pre-equilibrium is carried out with a 10-fold column-bed volume starting buffer, and the supernatant is injected into the Ni^{2+}-NTA affinity chromatography column.

3. Rinse with 10 ml starting buffer and collect filtrate.

4. Starting buffer containing 20 mM imidazole with 5 times column bed volume is used for elution and eluent is collected.

5. Starting buffers containing 40 mM, 60 mM, 80 mM, 100 mM, 200 mM, 300 mM and 500 mM imidazole are used for elution, and the eluents are

collected respectively.

6. SDS-PAGE is used to determine the optimal elution concentration of imidazole.

7. Take 10 ml sample from the filtrate collected in each tube, add 10 ml 2X SDS gel sample buffer, shake well.

8. Boiling water bath for 3 minutes.

9. Samples are taken out and all samples were electrophoresis on SDS polyacrylamide gel with appropriate concentration.

Note

1. Samples can be stored at 4℃ for addition to polyacrylamide gel.

2. Colmassie brilliant blue or silver staining is used to detect the purity of the recombinant protein, and the optimum elution concentration of imidazole is found, that is, the concentration corresponding to the eluent with the highest purified protein content; the purified protein is removed by PD-10 column.

3. After the protein has been eluted, the column should be cleaned in time so that it can be reused.

4. During the next purification of the same protein, the whole elution process can be optimized according to the protein concentration in each eluent shown on the gel map.