Protein purification using affinity chromatography

Protein Purification Using Affinity Chromatography

1. AB STRACT :

The principle behind this lab experiment was to purify the His-tag protein RNase H by implementing a technique called affinity chromatography. This technique is unique in its purification technological apparatus because it allows the purification of a biomolecule in accordance to its individual chemical disposition. A mini-column is prepared using 0. 5 mL of Ni-NTA agarose and washed with 10 mL of DI water. To begin the purification process, a sample of E. coli containing His-tag H is ran through a series of buffers to wash and remove unbound proteins, and then eluted to obtain the desired protein. During this procedure, the original flow through, eluting buffer flow through, washing buffer flow through and the unused eluting buffer will be retained and stored for the latter experiment.

2. INTRODUCTION :

Affinity chromatography is a technique for the purification of proteins. It isolates the transcription factors and purifys proteins by binding to a specific DNA sequence. The solution is passed down a column that contains the DNA sequence attached within the matrix. The proteins containing a relatively high affinity for the specific sequence are gravitated towards the matrix where it will remain and bind to the sequence. As given by the name itself, affinity chromatography is highly selective henceforth, superior resolutions and extreme capacity for proteins in query. Affinity chromatography isolates the proteins by means of a rescindable interaction linking the protein or in

some cases a group of proteins, and a distinctive ligand attached to a chromatographic medium. Affinity chromatography is an efficacious method when the interactions between the protein and the molecule of interest is highly specific.

However, the purification process can be a tad tedious and time-consuming. So to expedite the recovery of proteins while efficiently purifying recombinant proteins, affinity tags are introduced to various methods. The majority of the affinity tags are grouped as either a peptide or protein, which selectively adheres to the immobilized metal ion on the affinity column. The introduction of the affinity tags allows us to purify the proteins using affinity chromatography by taking advantage of the interaction associated with the metal ions and the protein molecules.

The affinity tag is the amino acid Histidine, called the His-tag. " The tagged proteins are passed through the column of beads containing covalently attached, immobilized nickel (II) or other metal ions" (Biochemistry, 2015). Histidine is known to display the greatest interaction with the immobilized transition metals, such as Ni^{2+}, therefore, they are the most commonly used affinity tag. This is due to the ionization property of the amino acid residue. Histidine contains an imidazole ring, that can bind and release protons depending on the surrounding environment of the matrix (Biochemistry, 2015). In this experiment, the matrix used for purifying the protein containing the His-tag is the Ni-NTA Agarose. The His-tag binds to the immobilized nickel (II) with great affinity and specificity, while the other proteins molecules are weakly bonded or end up getting washed out during the washing step.

The E. coli lysate is what is loaded into the minicolumn affinity matrix. The bound proteins remain attached while the other proteins wash through the matrix. After several washes, the bound His-tag protein is eluted from the column using an eluting buffer which will decrease the binding affinity and displaces the protein. The His-tag protein can also be eluted with imidazole, which is known to be the most generally used elution agent. In this experiment, the protein was purified by collecting the supernatant from each wash series which ran through a Ni-NTA affinity column. Each buffer contained different concentration values of Imidazole in increasing order, starting with 5 mM, 20 mM, and ending with 250 mM for the eluting buffer. The final flow through of eluting buffer wash contained the completed purified protein.

3. EXPERIMENTAL PROCEDURES :

MATERIALS:

- 0. 5 mL of E. coli lysate containing over-expressed His-tag RNase H
- 5 mL of Loading Buffer: 20 mM Tris-HCl, 0. 5 M NaCl, 5 mM Imidazole, & 10 % Glycerol
- 2. 5 mL of Washing Buffer (2x’s): 20 mM Tris-HCl, 0. 5 M NaCl, 20 mM Imidazole, & 10 % Glycerol
- 1 mL of Eluting Buffer: 20 mM Tris-HCl, 0. 5 M NaCl, 250 mM Imidazole, & 10 % Glycerol
- 10 mL of Glycerol (3x’s)
- 0. 5 mL Ni-NTA Agarose
- DI water
- HCl

- pH meter
- Mini-column

PROCEDURE :

Prepare the buffer solutions using the calculated values and adjust the pH with HCl until you reached a pH of…, and then top off to 100 mL with water.

**NOTE: The glycerol, Tris, NaCl, and imidazole can be added to 80 mL of water and the volume topped off to a total of 100 mL after the pH adjustment.

**Please refer to the data table for the appropriate values.

To prepare the minicolumn, add 0. 5 mL of Ni-NTA Agarose to the minicolumn and wash with 10 mL of DI water. Once the water has flowed through the column, add 5 mL of the loading buffer. When the loading buffer has gone through, proceed and add 5. 0 mL of the *E. coli* lysate and save the flow through for the following lab. Using the prepared washing buffer, wash the minicolumn twice with 2. 5 mL of the washing buffer and retain the flow through from the first wash only for the latter experiment. Once the washing buffer has completely flowed through the column, wash the minicolumn with 1 mL of the eluting buffer, and again save the flow through. The flow through from the eluting buffer was contains the final purified protein. Also, save 15 mL of the unused eluting buffer for the following lab experiment. Place all the saved flow through in the appropriate storing tube and label accordingly, including the initials of each group member or a distinctive marking so that it can be easily reclaimed in the next lab. Hand the labeled tubes over to the TA for proper storing, you should have a total of four solutions.

<u>DATA TABLE</u> :

To make 100 mL of Loading Buffer

20 mM Buffer	5 mM Solution	0. 5 M Solution	100 mL of a 10% Glycerol Solution	
Tris Needed	Imidazole Needed	NaCl Needed	Glycerol Needed	
<u>Loading Buffer</u>	0. 242 g	0. 3404 g	2. 922 g	10 mL

To make 100 mL of Washing Buffer

20 mM Buffer	20 mM Solution	0. 5 M Solution	100 mL of a 10% Glycerol Solution	
Tris	Imidazole	NaCl	Glycerol	
<u>Washing Buffer</u>	0. 242 g	0. 1362 g	2. 922 g	10 mL

To make 50 mL of Eluting Buffer

20 mM Buffer	250 mM Solution	0. 5 M Solution	100 mL of a 10% Glycerol Solution
Tris	Imidazole	NaCl	Glycerol

Eluting Buffer	0. 121 g	0. 851 g	1. 461 g	10 mL

4. REFERENCES :

1. J. M. Berg, J. L. Tymoczko, G. J. Gatto, Jr., & L. Stryer, *Biochemistry* (8th ed., pp. 70-71). W. H. Freeman & Company.
2. Hengen, P. N. (1995). Purification of His-Tag Fusion Proteins from E. coli. *Trends in Biochemical Sciences, 20* (7), 285-286.
3. https://www. qiagen. com/us/shop/sample-technologies/protein/expression-purification-detection/ni-nta-agarose/#orderinginformation
4. Biological Chemistry Laboratory Manual, (2017).