EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Editors

Ibrahim Ali Noorbatcha

Mohamed Ismail Abdul Karim

Hamzah Mohd Salleh

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CHAPTER 2

Protein Extraction and Purification

Faridah Yusof

1. Introduction

Scientists in biotechnology must face the challenge of protein purification regularly. But the question arises, why do scientists need to take the pain of purifying protein? In a research environment, proteins must be purified in order to:

- To analyze its function
- To analyze its physical properties
- To determine its amino acid sequence
- For industrial or therapeutic applications
- To develop an antibody to the protein

The degree of protein purity required depends on the intended end use of the protein. For some applications, a crude extract is sufficient. However, for other uses, such as in foods and pharmaceuticals, a high level of purity is required. In order to achieve this, several protein purification methods are typically used, in a series of purification steps. Each protein purification step usually results in some degree of product loss. Therefore, an ideal protein purification strategy is one in which the highest level of purification is reached in the fewest steps. The selection of which steps to use is dependent on the size, charge, solubility and other properties of the target protein.

2. Scope of This Chapter

This chapter is intended to be an introductory to protein extraction and purification research. The scope of this chapter is to present the common approach in protein purification consisting of development of assay for the protein of interest, extraction of protein from sources, fractionation of protein by a series of column chromatography steps and determination of protein purity. Emphasis was placed in the methods used in fractionating proteins by column chromatography. Altogether five methods of column chromatography are briefly discussed, namely ion-exchange, gel-filtration, hydrophobic interaction, chromatofocussing and affinity. Detailed discussion of all column chromatography methods can be easily be assessed from many text books, reviews and published journals. The author presented the methods of conducting column chromatography either on self-assembly low pressure system or on automated medium-pressure purification system by AKTA® Design supplied by GE Healthcare Lifesciences. An actual procedure of protein purification is also presented. In that aspect, an 'inhibitor' to rubber biosynthesis, also known as 'patatin-like protein' from *Hevea brasiliensis* latex was purified (Yusof *et al.*, 1998). By understanding the steps taken to purify the 'inhibitor', a researcher can then apply the knowledge to purify any protein of interest.

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