PREFACE

Protein purification is vital for the characterization of the function, structure and interactions of the protein of interest. This book presents current research from across the globe, including bacterial cellulose nanofibers for dye affinity carrier applications in protein adsorption systems; purification and applications of bacteriophage lytic enzymes; purification of novel sperm motility-related proteins; optimized purification protocol for proteins isolated from inclusion bodies; expression of proteins in E. coli, renaturation and purification by protein liquid chromatography and the purification of DNA-binding proteins.

Chapter 1 - Spermatozoa are highly specialised cells that possess flagellar motility which is essential for natural fertilization. Sperm motility measurement is associated with a serious technical problem due to sticking of these cells to the glass surface of haemocytometer required for motility analysis. The authors' research work has led to the discovery of novel anti-sticking factors (ASF-I and II) in caprine epididymal plasma (EP) that showed high affinity for inhibiting adhesion of spermatozoa to glass. These factors have been purified to apparent homogeneity by using multiple biochemical fractionation procedures. ASF-I and II are 47 and 36 kDa, heat-stable glycoproteins. The purified ASFs are suitable for eliminating the "cell-sticking" artefact in motility assays, as they have no role in sperm motility. Using ASFs in routine sperm motility assays, the authors have investigated biochemical basis of caprine sperm forward progression. A motility-promoting protein (FMSF) has been purified from buffalo serum using CM-cellulose chromatography, HPLC and native polyacrylamide gel electrophoresis. FMSF is a 66 kDa specific monomeric protein. Recently MIP: a motility initiating protein has been purified from EP. It is a 125 kDa dimeric protein made up of two subunits: 70 and 54 kDa. FMSF is also present in EP. Both the proteins serve as the physiological activators of sperm motility.

Studies from the authors' laboratory have identified for the first time a coupled ecto-enzyme system consisting of a protein kinase: CIK, a phosphoprotein phosphatase: PPase and their endogenous specific protein substrate: MPS, that controls the phosphorylation/dephosphorylation state of the endogenous cell-surface proteins, using spermatozoon as the cell model. All the protein components have been purified to apparent homogeneity from sperm membrane. Ecto-CIK is a dimer possessing two subunits: 63 and 55kDa. The CIK is a strongly basic protein. It is a unique membrane protein-specific serine/and threonine kinase. Ecto-MPS is a monomeric protein of 100 kDa. Both CIK as well as MPS are required for sperm forward motility and acrosomal reaction. Ecto-PPase is a monomeric 38 kDa protein.The coupled-enzyme plays pivotal role in regulating sperm

vigorous (vertical) movement. Sperm outer-surface also possesses a novel D-galactose-specific lectin and its receptor that have been purified by using Sepharose and Sepharose-lectin affinity chromatography, respectively. The lectin and its receptor have been implicated to play vital role in the regulation of sperm epididymal maturation and forward movement.

A motility inhibiting protein has been purified and characterized from sperm membrane. It is a 100 kDa monomeric protein. More recently another motility inhibitor (MIF) has been purified from EP using hydroxylapatite and DEAE-cellulose chromatograph and chromatofocusing. MIF is a heat-labile 160 kDa dimeric protein. A protein phosphatase (170 kDa) and a low molecular protein (14 kDa) have been identified in sperm cytosol and they are motility inhibitors. Sperm cytosol as well possesses a novel bicarbonate-activated soluble adenylyl cyclase that activates flagellar movement by elevating intra-sperm cyclic AMP level.

Some of these motility promoting proteins may be extremely useful for improving cattle breeding and breeding of endangered species of animals thereby helping in enhanced production of animal products as well as in the conservation of animals that are almost extinct. The motility-related proteins have also the potential for use as contraceptives to control population growth and for solving some of the problems of human infertility: a global problem of immense dimension.

Chapter 2 - Liquid chromatography (LC) is the most powerful method for the purification of proteins independent of sources. However, the purification of recombinant proteins from Escherichia coli (*E. coli*) is always required to refold target proteins in inclusion bodies before doing any subsequent purifications, bring in problems with not only long production technology, but also low bioactivity.

A new method called as protein folding liquid chromatography (PFLC) which can refold with simultaneously purify the inclusion body proteins is presented to solve this problem.

The PFLC is more flexible and is easy to achieve scale up, and its producing costs can be reduced exponentially. The main contents in this section include expression of proteins in E. coli and sample pre-treatment, practical range of PFLC, optimization of PFLC, new techniques in PFLC, new equipments and new methods, molecular mechanism of PFLC, application examples and future prospects.

Chapter 3 - With the increasing worldwide prevalence of antibiotic resistant bacteria, new antimicrobials are urgently needed. Bacteriophage (phage) endolysins are phage-encoded enzymes used for the release of the phage progeny from the bacterial host. In most cases they are peptidoglycan hydrolases which, when applied exogenously to Gram-positive bacteria, bring about rapid lysis and death of the bacterial cell. A number of studies have recently demonstrated the strong potential of these enzymes in human and veterinary medicine to control and treat various pathogens on mucosal surfaces and in systemic infections. They also have potential in bio-defence, elimination of food pathogens, diagnostics and detection. Paramount to the development of these enzymes as therapeutics is the knowledge and capability to produce them as highly purified recombinant proteins. This chapter reviews the different chromatographic purification techniques used to date in the study of endolysins, including ion-exchange, affinity, size exclusion and expanded bed adsorption. The authors also outline, in detail, two purification strategies, employed in their laboratory, in the development of endolysins, as purified recombinant proteins targeting strains of *Mycobacterium* and *Staphylococcus*.

Chapter 4 - Purified protein serves as a basic starting material for understanding its functions and for structural characterization. However, it is one of the most challenging steps

compared to all other proteomic techniques because of the fact that each protein differs in its characteristics in terms of chemical building blocks, i.e., amino acids, hydrophobicities, secondary and tertiary structures and functions. Thus, each protein or group of proteins requires a specific tailored purification procedure. This poses a need to develop either a new reliable and faster purification technique or to modify the existing protocols to suit similar groups of proteins. To obtain a purified protein of interest, the source can be from a natural system where protein is abundant or, in most cases, over-expressed protein using a bacterial or mammalian expression system. Once the source of the protein is understood, the purification procedure can be broadly classified into three categories, namely: cytoplasmic / periplasmic, membrane embedded and inclusion bodies-based on the localization of the protein. In this chapter, the authors discuss different purification procedures for the above mentioned classification and specifically, the proteins targeted to inclusion bodies, as in the case of Dengue virus (serotypes 1-4) envelope protein domain III. In particular, the problems encountered, such as low yield of histidine-tagged protein, aggregation, and removal of non-specific oligomers during the purification of dengue domain III protein, will be debated. The ways to overcome these problems in the course of purification procedures are highlighted. In addition, advantages and disadvantages of histidine-tagged protein purification using membrane, packed bed and His-trap columns are explained. Following purification, the methods used for protein refolding and the analysis of the protein by size exclusion chromatography to obtain monomers to homogeneity are also discussed.

Chapter 5 - DNA-binding proteins are fundamental elements in cells of all living organisms. They participate in essential cellular processes like DNA replication, transcription, recombination and repair, while some of them constitute valuable tools in routine genetic engineering manipulations. Nucleases, polymerases, topoisomerases, transcriptional factors and histones are some of the most common and best studied DNA-binding proteins, which can exhibit different domains that mediate binding to the nucleic açid including zinc finger, helix-turn-helix and leucine zipper motifs, among others. Although there is an important percentage of genes in the so far published genomes that code for DNA-binding proteins, their levels of expression are rather low in the cell. This hindrance can be overcome by producing higher amounts of recombinant protein instead of working with the original source. However, DNA-binding proteins usually work as dimers or multimers, making them prone to aggregate and difficulting their purification as soluble and functional products at a scale suitable for biochemical or structural studies.

This chapter focuses on the most common strategies developed for an efficient purification of functional DNA-binding proteins. Their isolation from native sources or their purification at larger scale as recombinant proteins require different approaches that are discussed.

Chapter 6 - The bacterial cellulose (BC) nanofibers were produced by *Acetobacter xylinum* in the Hestrin-Schramm medium in a static condition for 14 days. Then, Cibacron Blue F3GA (CB) was covalently attached onto the BC nanofibers for affinity adsorption of recombinant interferon-α (rHuIFN-α). The CB content of the BC nanofibers was 178 μmol/g. The specific surface area of the BC nanofibers was determined to be 914 m^2/g. rHuIFN-α adsorption studies were performed by batch system. The non-specific adsorption of rHuIFN-α on the BC nanofibers was very low (2.1 mg/g polymer). CB attachment onto the BC nanofibers significantly increased the rHuIFN-α adsorption (1620 mg/g). The maximum

rHuIFN-α adsorption was observed at pH 6.0. The rHuIFN-α adsorption capacity decreased drastically with an increase of the aqueous phase concentration of sodium chloride. The elution studies were performed by adding 1 M NaCl to the rHuIFN-α solutions in which adsorption equilibria had been reached. The eluted rHuIFN-α had a specific activity in the range of of 3.32-3.45 x 10^8 IU/mg as inhibition of the cytopathic effect of MDBK cells. In order to determine the effects of adsorption conditions on possible conformational changes of rHuIFN-α structure, fluorescence spectrophotometry was employed. The authors resulted that the BC-CB nanofibers can be applied for rHuIFN-α adsorption without causing any significant conformational changes. The elution results showed that the binding of rHuIFN-α to the BC-CB nanofibers was reversible. This study demonstrated that the BC nanofibers has a potential for affinity carrier applications in protein adsorption systems.