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BOOK REVIEW

Methods for Affinity-Based Separations of Enzymes and Proteins

(Munishwar Nath Gupta, ed., Birkhäuser, Basel-Boston-Berlin, 2002, 225 p.)

The book was written by a large group of authors from many countries and consists of 11 chapters that survey different aspects of methods for affinity-based separation of enzymes and proteins. This book is a volume of the well-known "Methods and Tools in Biosciences and Medicine" Series. The book is edited by Prof. Munishwar Nath Gupta, who is an unquestioned leader in this field.

In chapter 1, M. N. Gupta and I. Roy outline the principles of affinity-based separations (including precipitation, two-phase extractions, expanded bed chromatography, and perfusion chromatography) and list the different ways of exploiting affinity for separation purposes.

Chapter 2 (by E. Labrou) is devoted to affinity chromatography. Molecular recognition is considered as the basis for selective interaction of biological macromolecules. This chapter describes the methods of selection of ligand for affinity chromatography, ligand immobilization, and determination of immobilized ligand concentration. It is given basic procedures in affinity chromatographic separations (protocols) and outlined troubleshooting.

Chapter 3 (by A. Lali) is devoted to fluidized bed or expanded bed affinity chromatography as one of the promising methods that has the capability of handling large and delute crude extracts for adsorptive purification of proteins and enzymes in a speedy fashion. Some of the theoretical and practical aspects of expanded bed adsorption (EBA) technology are discussed. In particular, different types of base adsorbents are discussed and general methods of column preparation and several purification procedures for proteins are given.

In chapter 4, K. L. Fahrner and G. S. Blank consider perfusion affinity chromatography for rapid antibody and antibody fragment separations. Perfusion chromatography media are polymeric and contain large through-pores that completely penetrate the chromatographic bead. These large pores allow convective (or perfusive) flow through the bead. Along the large pores are also smaller diffusive pores with short diffusive path lengths. The combination of convection and diffusion allows perfusion chromatography to be run at extremely high flow rates and high level of purification. The authors describe the use of rapid affinity chromatography for both analytical and preparative applications.

Chapter 5 (by L. G. Berruex and R. Freitag) presents new developments concerning affinity-based interactions on disks for fast analysis, isolation, and conversion of biomolecules. This is still a developing technique. The chapter describes general methods for immobilization of biomolecules, properties of disks, and their application for affinity chromatography and biocatalysis, e.g., cleavage of peptides by immobilized chymotrypsin or trypsin. Some protocols as well as optimizations of some existing procedures are given.

Chapter 6 (by P. R. Satish and A. Surolia) is devoted to sugars as affinity ligands. Protein–carbohydrate recognition is powerful tool for isolation of carbohydrate-binding proteins in general and of lectins in particular. General methods of direct coupling of sugar to activated agarose, coupling of sugar to activated agarose using a spacer, and preparing aminoethylacrylamide gels for the purification of lectins cross-linking of polysaccharides as affinity matrices are described. Specific protocols for immobilizing sugars and/or their derivatives on various matrices, cross-linking of matrices and their schemes, followed by their application to lectin purification, general solution to problems that could be encountered are also discussed.

Chapters 7 (by I. Roy and M. N. Gupta), 9 (by A. Kumari et al.), and 10 (by M. Adachi) outline a brief review of the principles and protocols related to the non-chromatographic affinity-based separations. All the macroaffinity ligands described in chapter 7 belong to a class of materials called smart polymer and form the basis of a technique called affinity precipitation. This is affinity partitioning combining the capability of biological macromolecules to partition in aqueous two-phase systems with the principle of biorecognition (chapter 9). Affinity-based reverse micellar separations are discussed (chapter 10). Such approaches have been possible with specially tailored media, which are capable of being conjugated to various affinity ligands.

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Chapter 8 (by A. B. Boraston et al.) is devoted to cellulose binding fusion proteins. New developments are described concerning the use of cellulose binding domains (CBDs) as affinity tags to exploit the attractive properties of cellulose as a purification affinity matrix or insoluble support for protein immobilization. Different CBDs exhibit different binding specificity and desorption characteristics. CBDs are discrete polypeptide domains found in proteins, such as cellulases, that mediate tight binding to cellulose. CBDs can be coupled by chemical or molecular genetic techniques. Binding to cellulose is rapid, stable and, under appropriate conditions it can be reversed.

The last chapter 11 (by A. Ramakrishnan and A. Sadana) considers a new area in the investigation of biological macromolecular interactions: development of biosensors. In particular, biosensors can be used to monitor analyte–receptor reactions in real time. Some techniques like surface plasmon resonance (SPR) biosensor in combination with mass spectrometry exhibits the potential to provide proteomic analysis. In addition to described reactions on surfaces, this approach can be further extended to describe reactions occurring on cellular surfaces, DNA hybridization kinetics, drug–receptor interactions, etc.

Consequently, this book is very useful for specialists working in biochemistry, biotechnology, and biochemical engineering. The book is valuable for students and graduate students. The book is well illustrated, and each chapter contains a bibliography. A useful index will help reader to find desired information.

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