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CHAPTER 9

THE APPLICATION OF HPLC FOR PROTEINS

Kálmán Benedek

1. Introduction

The 1980s witnessed the growing pains and maturing of a new technology in pharmaceutical discovery and development, the emergent biotechnology industry. The spectrum of drugs has been extended from the traditional 'small' molecules to peptide- and protein-based pharmaceuticals. Biotechnology has become a practical and feasible possibility for medicine. The technical cornerstones of this new era are the three C's; cloning, computer and chromatography. Chromatographic techniques are implemented for the purification and characterization of all proteins, from basic discovery research to full product development [1]. High performance separations are required to isolate a given protein from the complex matrix of cell culture media, and are involved in verifying the purity and identity of the product and in monitoring its stability.

HPLC is the most versatile separation technique so far, because the acronym 'HPLC' includes such mechanistically diverse chromatographic methods as hydrophobic–interaction chromatography (HIC), reversed phase liquid chromatography (RPLC), electrostatic interaction chromatography (EIC), size exclusion–chromatography (SEC), metal-interaction chromatography (MIC) and biospecific chromatography (BIC) or affinity. The use of so many HPLC separation modes is required because of the diversity of proteins. In order to apply successfully the techniques of modern HPLC,

the underlying phenomena should be understood and taken into consideration. Protein chromatography is a multidisciplinary science in which protein biochemistry, polymer adsorption, surface chemistry and chromatographic theories have to be simultaneously considered during the development of each separation method. In the following chapter some of the relevant phenomena associated with protein chromatography will be highlighted, with special attention to adsorption, conformational effects and their impact on chromatography. The examples were selected according to criteria which could best help to illustrate the most frequently occurring problems and to focus on significant accomplishments of protein chromatography.

2. Protein Composition and Structure

Compared with the challenges of the analysis of 'small' molecules, most of the technical difficulties of protein chromatography are the direct consequence of the polymeric, macromolecular nature of proteins. Proteins are linear polymers of up to 21 different amino acids, with a molecular weight ranging from a few to a few hundred kilodaltons. Each particular protein has its characteristic amino acid sequence, called the primary structure. Intermolecular forces between the amino acids of the linear polymer generate local helices, sheets and turns, called secondary structure. The folding of the secondary structure into a three-dimensional entity produces a specific biological activity, and is called the tertiary structure of a protein. In general, only one folded conformation can be assigned to the native protein. A number of proteins have a quaternary structure, which is the association of folded polymer units into a biologically relevant structure.

Protein structures can further be divided into two major classes on the basis of their composition: simple and conjugated. Simple proteins are linear polymers of the common amino acids. Conjugated proteins have other organic and/or inorganic components attached through the side chains of particular amino acid residues. The attached moieties are called prosthetic groups. Conjugated proteins can be classified on the basis of the chemical nature of their prosthetic groups (*e.g.* nucleoproteins, lipoproteins, phospho-

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