
Preface

Amino acid analysis is a technique that has become commonplace in biotechnology, biomedical, and food analysis laboratories. This book describes a variety of amino acid analysis techniques and how each technique can be used to answer specific biological questions.

The first two chapters in *Amino Acid Analysis Protocols* introduce the concepts, basic theory, and practice of amino acid analysis. The following chapters give detailed instructions on various methods and their applications.

As highlighted, there are many different approaches to amino acid analysis, but in all cases the results depend heavily on the quality of the sample. Therefore a new way to desalt samples prior to hydrolysis is covered as an introductory chapter (Chapter 3), and most authors have devoted a section to sample preparation, especially to the collection and storage of bodily fluids.

Some of the amino acid analysis methods described in this book are based on HPLC separation and analysis after precolumn derivatization. The precolumn derivatization techniques described use (a) 6-aminoquinolyl-*N*-hydroxy-succinimidyl carbamate (AQC) (Chapters 4 and 8); (b) 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent), which allows separation and analysis of enantiomeric amino acids (Chapter 5); (c) *O*-phthalaldehyde (OPA) (Chapters 6 and 10); (d) butylisothiocyanate (BITC) and benzylisothiocyanate (BZITC) (Chapter 11); (e) phenylisothiocyanate (PITC) (Chapters 12 and 13); (f) ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) (Chapter 17); and (g) 9-fluorenylmethyl-chloroformate (Fmoc-Cl) (Chapter 10).

Techniques have been described in which gas chromatography is used to separate and analyze (a) amino acids after *N*(*O,S*)-isoBOC methyl ester derivatization (Chapter 9); (b) *N*-isoBOC methyl esters of *O*-phosphoamino acids (Chapter 14); and (c) *N*(*S*)-isopropoxycarbonyl methyl esters derivatives of sulfur amino acids, glutathione, and other related aminothiols such as CysGly (Chapter 15). New techniques based on capillary electrophoresis separation (Chapter 16), high-performance anion-exchange chromatography (Chapter 7), and mass spectrometry of isotopically labeled proteins (Chapter 18) are also presented.

The applications of amino acid analysis are extremely varied and the technique remains the best means of accurate protein quantitation. Examples given in *Amino Acid Analysis Protocols* include the use of amino acid analysis for identification of picomolar amounts of protein on PVDF membranes (Chapter 8). The measurements of amino acids in bodily fluids and tissues such as urine (Chapters 9, 12, 14, 15), blood (Chapters 9, 10, 12, 14, 15, 17), seminal plasma (Chapter 6), or skeletal muscle tissue (Chapter 16), and measurement in the presence of high lipid content, such as in porcine lung (Chapter 13), are useful to help to identify diseases associated with changes in amino acid metabolism. Amino acid analysis, for example, is important to the study of such disorders as maple syrup urine disease (accumulation of branched-chain L-amino acids), phenylketonuria (high concentrations of phenylalanine), atherosclerosis (elevated levels of homocysteine), and galactosemia (often high concentrations of methionine). Amino acid and glucose analysis in fermentation broths of cell cultures (Chapter 7) enables the development of a feeding strategy that maintains the correct levels of nutrients. This is important since the use of such systems to make recombinant products is increasing. A method to determine the amino acid composition of foods (Chapter 11) is also included.

In addition to the standard methods used to separate the 20 commonly occurring amino acids, the analysis of unusual and modified amino acids is also addressed. Specifically, the analysis of homocysteine for monitoring the development of atherosclerosis (Chapter 17); hydroxyproline, a major amino acid found in collagen (Chapter 16); phosphoamino acids, which are difficult because they are acid labile (Chapter 14); aminothiols, such as cysteinylglycine and cystathionine (Chapter 15); and glycated lysine, implicated in diabetic complications and Alzheimer's disease (Chapter 18).

Overall *Amino Acid Analysis Protocols* presents an up-to-date, detailed methodology reference for a broad range of current techniques being used for amino acid analysis.

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