ACCELERATION AND IMPROVEMENT OF PROTEIN
IDENTIFICATION BY MASS SPECTROMETRY

Acceleration and Improvement of Protein Identification by Mass Spectrometry

Edited by

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"Conformity is the jailer of freedom and the enemy of growth."

John F. Kennedy

DEDICATION

To my parents for their help and support during all the years.

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PREFACE

Now that the human genome has been fully sequenced, the need for efficient protein analysis and characterization tools has never been so critical. Firstly, computer algorithms have been used to predict genes and it is accepted that as much as 10% of them might have been missed. Only final gene products, i.e. the proteins, prove that gene sequences with signal sequences, introns and exons are correct. Secondly, it is nearly impossible at present to predict with high accuracy the final polypeptide product and its co- and post-translational modifications. Then, a protein's partial characterization allows a definite identification of the protein's processing such as the amino-acid sequence modification induced by the editing of the mRNA during alternative splicing. Thirdly, there is lack of correlation between the expression levels of mRNA and proteins. Their respective half-lives are very different as well as their levels of expression.

The major difficulty in analysing proteins is the tremendous diversity of their chemical and other properties. Their concentrations vary by more than 12 orders of magnitude in body fluids and by more than 7 orders of magnitude in cells. For example in blood, the concentration of albumin is in the millimolar range and Tnf (tumour necrosis factor) in the femtomolar range. While the pI (isoelectric point) of DNA or mRNA is around 4.2 to 4.5, the pI of proteins extends from less than 3 to more than 12. Whereas the solubility of nucleic acids is excellent, proteins, and especially membrane proteins, can be excessively hydrophobic. Consequently, no single method is available to fully analyse a complex mixture of proteins. In addition, no amplification process such as PCR or RT-PCR exists in the protein world. Therefore, extremely sensitive methods are required to detect the low-abundance proteins.

Many methods to separate, identify and partially characterize polypeptides have been available for a long time. Until recently, many of them required a relatively high concentration/amount of proteins/material. Miniaturization of the analytical techniques does not necessarily solve the difficulty in detecting low-abundance proteins. For example, if in equipment with attomole sensitivity one injects a volume of nanolitres, the limit of detection in concentration does not exceed micromolar, way above the concentration of interesting physiologically relevant proteins. Consequently, the critical step in working with complex protein samples is to select efficient pre-fractionation and separation techniques. Often the best methods are based on affinity pre-purification and a combination of chromatography and/or electrophoresis. Many approaches could be used to detect the proteins and some of their modifications.

Several developments in the field of mass spectrometry offer a new avenue, especially in the area of large-scale protein identification and partial characterization. Multi-compartment equipment allows the precise selection of

precursor ions (peptides), their efficient fragmentation and final characterization (sequence and modifications) and can also provide accurate quantification methods. The latest improvements are at both the hardware and software levels to provide fully automated and rapid identification methods.

This book is timely. It reviews in a concise form most techniques that should be known by scientists working in a proteomics laboratory or analysing proteins of interest. It first reviews the electrophoretic and chromatographic separation methods. It then summarizes the quantification and identification methods such as immunoblotting, protein chemistry, peptide fingerprinting or sequencing by fragmentation. Several chapters highlight fascinating developments in the field of mass spectrometry and related techniques. The text shows the reader the perspective of this relatively new field of proteomics.

Finally, the book lists numerous references to critical work done many years ago and unavailable on computer databases. It should therefore be part of every laboratory's library.

Prof. Denis F. HOCHSTRASSER

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