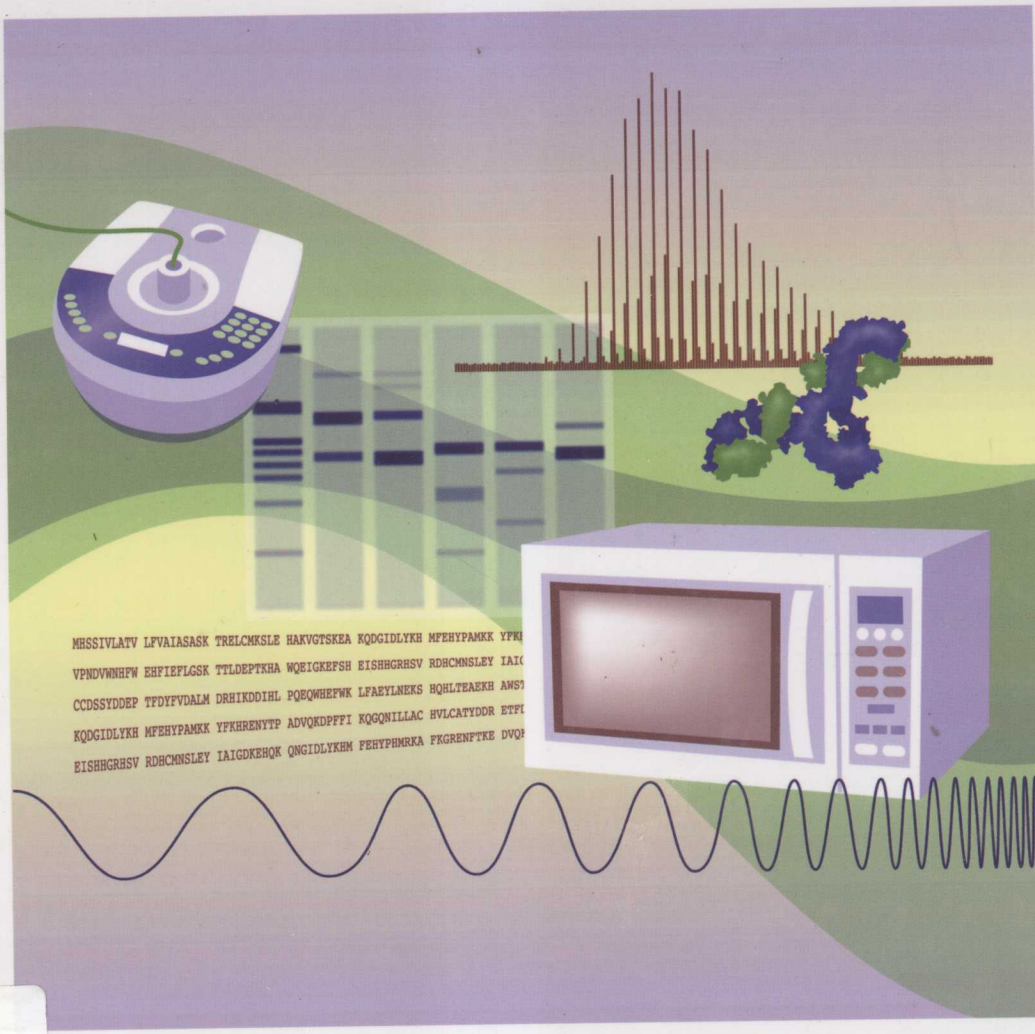
Jennie Rebecca Lill

Microwave Assisted Proteomics



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Microwave-Assisted Proteomics

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Microwave-Assisted Proteomics

Dedication

To my wonderful husband Glen and my beautiful son Joseph.

Preface

Since the conceptualization of the electromagnetic spectrum and development of the magnetron, microwave energy has been utilized in many aspects and disciplines of science. Although adopted by multiple industries over the past quarter of a century, it is only within the past few years that microwave irradiation has been evaluated as a useful tool for the biochemical and chemical preparation of proteins and other biomolecules for proteomics and in particular mass spectrometric analysis. This book describes the evolution and integration of microwave energy into the biosciences with particular emphasis on the proteomic arena. An in-depth evaluation of a variety of techniques within the field of proteomics that benefit from microwave irradiation is given. This book chronicles the development of these microwave-assisted methods and provides a synopsis of the final protocols that have become standardized for each area discussed. This book also focuses on the types of instrumentation that may be employed for microwave-assisted protein chemistries and the hypotheses of mechanisms of action for the microwave-enhanced methodologies.

Although still in its infancy, the application of microwave assistance is gaining momentum in several fields, in particular that of proteomics and protein chemistry. A single monograph cannot comprehensively cover this rapidly evolving field; however, this publication should provide an in-depth introduction to the science of microwave-assisted proteomics and will hopefully ignite some ambition within researchers interested in trying these protocols in their own laboratories.

Jennie R. Lill PhD

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CHAPTER 1

Evolution of Microwave Irradiation and Its Introduction to the Biosciences

Abstract

Since the conceptualization of the electromagnetic spectrum, through the development of the magnetron microwave energy has been utilized in many aspects and disciplines of science. Although adopted by many industries over the past quarter of a century, it is only within the past few years that microwave irradiation has been evaluated as a useful tool in the biochemical and chemical preparation of proteins and other biomolecules. This chapter describes the evolution of the magnetron and some early applications of microwave assistance in the bioanalytical sciences.

1.1 Microwave Radiation

The electromagnetic spectrum is a continuum of all electromagnetic waves arranged according to frequency and wavelength. Microwaves occupy the electromagnetic spectrum between infrared radiation and radio waves and have wavelengths between 0.01 and 1 m with a frequency range between 0.3 and 30 GHz (Figure 1.1). Most commercially available microwaves have a narrower range at around 2.5 GHz.[1] Microwave energy is a natural phenomenon which can be induced when electric current flows through a conductor, for example an antenna, a transmitter chip or a magnetron.

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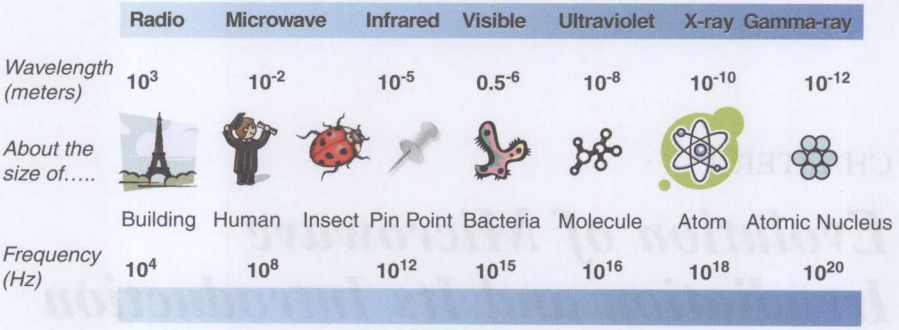


Figure 1.1 The electromagnetic spectrum modified from http://en.wikipedia.org/wiki/Electromagnetic_spectrum. Microwaves reside in between radio waves and the infrared portion of the electromagnetic spectrum.[2]

1.2 History and Evolution of the Magnetron

The theory of microwave irradiation was first documented in 1864 by James Clerk Maxwell, a Scottish mathematician and theoretical physicist. In the eponymous Maxwell's equations he described a unified model for electromagnetism and paved the way for modern physics.[3] It was not until over twenty years later that microwave irradiation was physically demonstrated by Heinrich Hertz.[4] Hertz was a German physicist who demonstrated the existence of electromagnetic radiation by building antennae and apparatus to produce and detect high-frequency radio waves. Microwaves were produced using various apparatus up until the 1920s when Albert Hull, a researcher at General Electric's research laboratories, invented the simple two-pole magnetron, or split-anode magnetron.[5] Although revolutionary at its time, the two-pole magnetron was relatively inefficient and was soon superseded by the resonant-cavity magnetron which proved to be more efficient and convenient.

The first half of the twentieth century become synonymous with large-scale war and scientific innovation, the combination of which led to the development of many technologies including the introduction of radar. In a deal between British and American researchers, the cavity magnetron was developed into a viable radar system, and by 1941 magnetrons for radar systems were being manufactured at a rate of 17 per day at Raytheon. It was during this time that a researcher at Raytheon, Percy Lebaron Spencer, made two important discoveries. Firstly, he was awarded the Distinguished Public Service Award by the US Navy for significantly improving the manufacturing process of magnetrons and increasing production more than 100-fold. Secondly, perhaps more famously, in 1945 while standing in front of an open magnetron he noticed that a chocolate bar had melted in his pocket. After several other "tests" including popping popcorn and exploding eggs he concluded that microwave radiation could be tailored for use in cooking devices and hence the invention of the microwave oven.[6,7]

By 1947 the first commercial microwave oven had been manufactured by Raytheon, although during the first few years of commercialization these ovens stood at nearly 6 feet tall and weighed over 700 pounds. By the 1970s microwave ovens had become much more accommodating for household use, and by 1975 the sales of microwave ovens started to exceed those of gas oven ranges in the USA.

In 1978 the first commercial microwave for laboratory use was introduced by CEM and, since then, laboratory microwaves have increased in sophistication and utility to include models specific for the chemical and biological sciences. Figure 1.2 shows the time scale of the evolution of the magnetron.

1.3 Microwaves as a Catalysis Tool in Organic and Inorganic Chemistry

In 1986 the first reports of high-speed chemical synthesis with microwave assistance were published.[8] Since then there have been numerous publications describing microwave-assisted synthetic reactions where most researchers observe shorter reaction times, increased yields and cleaner syntheses due to reduced by-product formation or side reactions (for a comprehensive review, see Alcazar *et al.*[9]). Originally, chemists would employ microwave assistance only for those reactions that proved troublesome or that resulted in poor yields. Nowadays, however, as instrumentation and an understanding of the mechanisms involved have matured, chemists routinely employ microwave-catalyzed protocols at the first stage of method development. Indeed for reactions involving highly polar reagents or metal catalysis, microwave irradiation is confirmed as the most valuable mode of heating available, and standard protocols for polymer synthesis and process control typically employ microwave assistance.[10–12]

1.4 Microwave-Assisted Staining of SDS-PAGE and PVDF Membrane-Embedded Proteins

One of the first microwave-assisted applications in the biological setting was the fixing, staining and destaining of sodium dodecyl sulfate polyacrylamide gels and poly(vinylidine difluoride) (PVDF) membranes. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a highly valuable technique employed in biochemistry, genetics and molecular biology to separate biomolecules according to their electrophoretic mobility. In the separation of proteins, the electrophoretic mobility is dictated by the length of the polypeptide chain or molecular weight as well as higher order protein folding, posttranslational modifications and other factors. After proteins are separated on the gel, the sample is typically fixed with a reagent to mobilize the gel and to stop migration or dispersion. Fixation is typically performed in a high percentage of methanol,

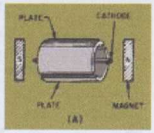
Raytheon

1864

James Clerk Maxwell described a unified model for electromagnetism and therefore was the first to describe microwave irradiation.

1880's

Heinrich Hertz demonstrated electromagnetic radiation by building antennae and apparatus to detect high frequency radio waves.



1920's

Albert Hull invented the two-pole, or split-anode magnetron.

1941

Radar was developed as an essential tool for detecting enemy planes during WWII.

1945

Percy Lebaron Spencer, while working on Radar at Raytheon, discovered that the magnetron could be used to heat food. This first microwave was known as "the radar range"

1980's

The Laboratory application microwave is developed. Chemists start to use microwave-assistance to catalyze reactions producing higher yields, less side products and quicker reactions.

2000's

Microwave-assistance enters the biological arena.

Figure 1.2 A time line of important events in the history of microwave energy discovery through its evolution as a tool for analytical biosciences.

which can clean up the gel from any remaining material from the SDS running buffer. After fixation the gel is stained using one of the many stains available, for example Coomassie Blue or silver stain. Traditional staining protocols recommend immersion of the gel or membrane into stain solution for many hours (often an over night incubation). After staining, gels are destained to remove background stain, and to allow the bands corresponding to the proteins of interest to be visualized. Proteins may also be electro blotted from the gel onto PVDF membranes whereby the sample is more compatible with long-term storage and with certain analytical techniques, for example Edman degradation. PVDF membranes can be stained and destained in the same manner as SDS-PAGE.

Microwave irradiation has been successfully employed to speed up the process of fixation, staining and destaining since the early 1990s; however, the first citation on record appears to be that of Nestayy *et al.* Here an in-depth study into the effect of microwave irradiation on the staining of proteins in gels or membranes using a variety of common stains was performed.[13] Microwave-assisted staining with Coomassie Blue, SYPRO® Ruby, silver stain and colloidal gold protocols was evaluated. Nestayy *et al.* demonstrated that the traditionally time-consuming process of staining and destaining gels could be significantly reduced if performed in the presence of microwaves. A regular domestic microwave oven was used and gels were introduced into the cavity of the microwave in Petri dishes or any microwavable container. It was postulated that the faster staining and destaining was mainly due to heat produced by the microwaves which maximized the efflux and influx of solvent and solutes from the gel or membrane.

After separation of proteins by SDS-PAGE, proteins were identified by performing an in-gel tryptic digestion followed by tandem mass spectrometric characterization (see Chapter 4). Nestayy *et al.* went on to monitor the effect microwave-assisted staining had on subsequent mass spectrometric analysis. Increased proteolytic cleavage was observed after microwave-assisted staining compared to conventional methods (*i.e.* room temperature incubation). It was proposed that this effect was due to increased denaturation of proteins embedded in the matrices of the gel or membrane after microwave exposure. (Note that the digestion itself was not performed in the presence of microwave radiation, only the staining and destaining of the gel or membrane.) This denaturation resulted in greater accessibility of the substrate proteolytic sites to the enzyme. Heat generated from the microwave process may also have contributed to gel or membrane expansion, therefore enhancing exposure of the protein to enzymatic cleavage, and also resulting in increased extraction of peptides from the gel or membrane after digestion.

Overall it was concluded that microwave irradiation of proteins separated by SDS-PAGE or blotted onto PVDF membranes often significantly improved proteolytic coverage when compared to traditional gel-staining techniques. In addition, there did not appear to be any detrimental effects such as loss of posttranslational modifications or increased deamidation or oxidation. For a practical protocol on microwave-assisted fixation, staining with Coomassie Blue and destaining of gels, refer to protocol I in Chapter 10.

A microwave-enhanced ink staining method was also recently reported whereby dye-based blue-black ink was used to quantitatively visualize proteins spotted onto a nitrocellulose membrane by incubation in a domestic microwave oven. The total staining time was reduced from more than 30 min to less than 3 min by employing microwave assistance. A 500-fold dynamic range from low nanogram to mid-microgram total protein amounts could be detected using this method, which in addition allowed samples in complex buffers and chaotropes to be quantified.[14]

1.5 Microwave-Assisted Peptide Synthesis

Peptides may be synthesized for a number of reasons including internal standards in mass spectrometric quantitation studies, synthetic hormones or neurotransmitters, for mapping enzyme specificity or drug interaction sites and other bio-pharmacological tools. Chemical synthesis of peptides is often preferred to *in vivo* or *in vitro* synthesis as samples are free from other cellular debris such as contaminating peptides, lipids or genetic material. In addition, chemical synthesis allows flexibility in the design of the peptide and allows incorporation of specific modifications or isotopic and other non-natural amino acids into the peptide backbone.

Microwave irradiation as a tool for peptide synthesis was first described in 1992,[13] and since then has been applied to thousands of peptides, some composed of up to as many as 200 amino acid residues. Microwave-assisted peptide synthesis has many advantages over conventional solution- and solid-phase protocols including higher yields, higher specificity during coupling, higher deprotection and less racemization.[15] Although the exact mechanisms of microwave assistance are not proven, the main hypothesis is driven by evidence that, during synthesis using conventional methods, the reaction matrix can be rendered inaccessible leading to aggregation with both itself and neighboring peptides. Microwave irradiation may lead to deaggregation *via* dipole alignment, allowing increased accessibility of reagents and hence more efficient deprotection, coupling and washing.[16,17]

1.6 Microwave-Assisted Antigen Retrieval

Immunohistochemistry or immunohistochemical staining is the process whereby tissues, cells or proteins are localized using antibodies raised against antigens of interest. Typically this is accomplished using either a primary antibody or combination of a primary and secondary antibody that is conjugated to a visualization tool, for example a fluorophore or fluorescent molecule (refer to Chapter 8 for more microwave-assisted immunoassays). Many samples, for example tumor biopsies, requiring immunohistochemical staining are fixed in formalin or paraffin embedded for preservation, treatments which are not conducive to antigen exposure. To increase antigen exposure (a protocol known