

# Electron Paramagnetic Resonance in Enzymology

Aimin Liu, *Department of Chemistry, Georgia State University, Atlanta, Georgia*

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Advanced Article

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Electron paramagnetic resonance (EPR) is a versatile tool for chemical biology research. A remarkable wealth of information about the mechanistic enzymology has been obtained by EPR experiments. As an analytical method, EPR can obtain these two useful pieces of information in a study of enzymatic reaction mechanisms: 1) detection and complete description of free radicals and 2) characterization of the electronic structures of paramagnetic metal ions and their response to changes of the protein environment or substrate binding. The first feature has spurred the development of the new field known as radical enzymology; the second has enhanced the understanding of the mechanisms of metalloenzyme action. The potential of EPR spectroscopy to serve as an important biophysical tool for the future development of enzymology is highlighted by several selected examples.

## Introduction

This article does not intend to be a survey of electron paramagnetic resonance (EPR) spectroscopy in chemical biology, but rather the focus is to provide an entry-level introduction to chemists who are interested in the research opportunities that EPR may provide for enzymology studies. The principles of this technique will be briefly described, as well as the breadth of the technique's applicability to free radicals and metallocenters in enzymology. Potential applications in the future will also be outlined.

## History

EPR was first applied to biological materials in 1954 (1), 10 years after the discovery of an experimental approach to detecting electron spin resonance by the Russian physicist Zavoisky in 1944 (2, 3). This technique became useful for studying enzymes when a spectrometer that was capable of detecting about  $10^{-10}$  moles of unpaired (nonbonding or "free") electron spins in samples that contained about 0.15 milliliter of liquid water was developed by Townsend and colleagues in 1957 (4). The first successful application of this technique to elucidating the structure of substrate radicals in enzymatic reactions was in 1958 (5–7). In the ensuing 50 years, numerous seminal and classical contributions have been made to the development of enzymology by scientists employing EPR spectroscopy analytically.

## Application

It is an advantage to analyze EPR samples of aqueous solution directly because the buffered solution is the most physiologically relevant condition. However, EPR samples are not restricted to samples in the solution state, gaseous and solid samples and single crystals can also be analyzed directly by EPR spectroscopy. The only absolute requirement for preparing an EPR sample is that it must contain unpaired electron spins or that such status can be achieved before EPR measurements by oxidation/reduction reactions. EPR spectroscopy can determine unambiguously free radicals that are present in the sample or radicals produced by oxidation or irradiation with light, X-rays, and  $\gamma$ -rays. Because paramagnetic transition metals contain unpaired electrons, EPR spectroscopy can determine the detailed geometric and electronic environment of the metal centers. This technique can also indicate the degree of molecular motion in a sample with unpaired electrons and analyze the populations in a heterogeneous sample that has two or more paramagnetic species. Its ability to focus on the paramagnetic active sites without interference from the rest of the diamagnetic species makes this technique an ideal method for mechanistic studies. Therefore, it is commonly employed in studies of the structure of enzyme active site, interactions between enzyme and substrate, conformational dynamics, electron transfer, and reaction kinetics.