RAPID-FREEZE-QUENCH EPR: WHAT, WHY AND HOW

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The generation of samples for the study of reaction kinetics by quenching a chemical or enzymatic reaction after appropriate time intervals was described by Roughton & Chance in 1953 (1). Bray introduced the method of quenching by rapid freezing via mixing with a cold immiscible solvent in 1961 (2), and adapted the method specifically for EPR investigation of the samples (3). The method was subsequently applied to the EPR analysis of reaction intermediates in the iron-and molybdenum-containing metalloenzyme xanthine oxidase (4); this study was the first example of RFQ-EPR 'spectrokinetics', so called because both spectroscopic and kinetic information are available from the same experiment. Refinements in the methodology (5), mixing and freezing devices (6,7), and sample collection (8) have improved absolute spin quantitation and lowered the 'dead-time' from ~ 10 ms to ~ 50 μs. Wide access EPR cavities (e.g. Varian E-235) allow for EPR examination of RFQ samples prepared for other techniques, such as resonance Raman or XAS. RFQ-EPR has most commonly been applied to metalloenzyme reactions. Time dependent changes in spectra can report on metal ion coordination changes during a reaction cycle (9), and intensity changes can report on the oxidation state of a metal cofactor during redox reactions or electron transfer. Using 'parallel-mode' EPR (B₀∥B₁), reaction-dependent changes in spin coupling in a multinuclear site can also provide mechanistic information (10). Aside from catalytic reactions, RFQ-EPR has found utility in the study of metal binding and metal cofactor assembly in metalloenzymes (11). More recently, RFQ has been of value as a technique for the investigation of protein conformational changes during protein folding (12) or during a catalytic cycle (13).

The principles of, the rationale for, the practice of, and opportunities afforded by RFQ-EPR will be presented from a practitioner's perspective.

References: