

Ribonucleotide Reductases: Use of Unnatural Amino Acids to Understand the Radical Initiation Process.

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Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to deoxynucleotides in all organisms playing an essential role in DNA replication and repair. The class I RNRs are composed of two homodimeric subunits: R1 and R2. R2 contains the di-iron Y• cofactor essential for activity. R1 contains the active site where nucleotide reduction occurs and the effector sites that control the specificity and rate of nucleotide reduction. An unresolved mechanistic issue is how the Y• on R2 generates a transient thiyl radical on R1 to initiate the reduction process. This initiation is thought to occur over a 35 Å distance and involve generation of aromatic amino acid radical intermediates. Direct evidence for the long distance is provided by PELDOR experiments. Experiments using unnatural amino acids ((F)_n-tyrosines (F = 2, 3, 4), dopa, and benzophenone) in position 356 of R2 will be described. The DOPA-R2 experiments trap the radical at 356 only in the presence of R1, substrate and effector. The studies with the (F)_n-tyrosines derivatives provide direct evidence for the redox activity of 356. Thus our recent studies provide direct evidence for hole migration through amino acid radical intermediates.