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Radicals with a controlled lifestyle

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JoAnne Stubbe discusses the importance of stable and transient amino acid radicals in primary metabolism.

Introduction

One cannot open a newspaper or magazine without reading about aging and death and usually close behind are the putative culprits blamed for these inevitabilities—free radicals. Vitamins E and C, popular items in the health store scene, are claimed to be life-extenders, as they reduce free radicals and the damage associated with these species. In general, free radicals are vilified. They are associated with uncontrollable reactivities leading to mutations in DNA and consequently proteins. It will thus come as a surprise to many scientists, that a number of primary steps in metabolic pathways involve protein-based radicals.[1] Nature has evolved methods to specifically generate these amino acid radicals within a protein environment and to exquisitely control their reactivity and life-times in order to avoid their potential promiscuities.

Abbreviations

RNR, ribonucleotide reductase
Y·, tyrosyl radical
PSII, photosystem II
EPR, electron paramagnetic resonance
ENDOR, electron nuclear double resonance
HF, high frequency

The first amino acid radical was discovered in ribonucleotide reductase (RNR) by Ehrenberg and Reichard in 1972[2] and its structure was assigned to that of a neutral tyrosine radical (Y·, Fig. 1A) by Sjöberg *et al.* in 1978.[3] Since this discovery, a broad range of amino acid radicals within proteins have been identified and shown or postulated to play central roles as cofactors in many chemical transformations encountered in metabolism (Fig. 1). The two most

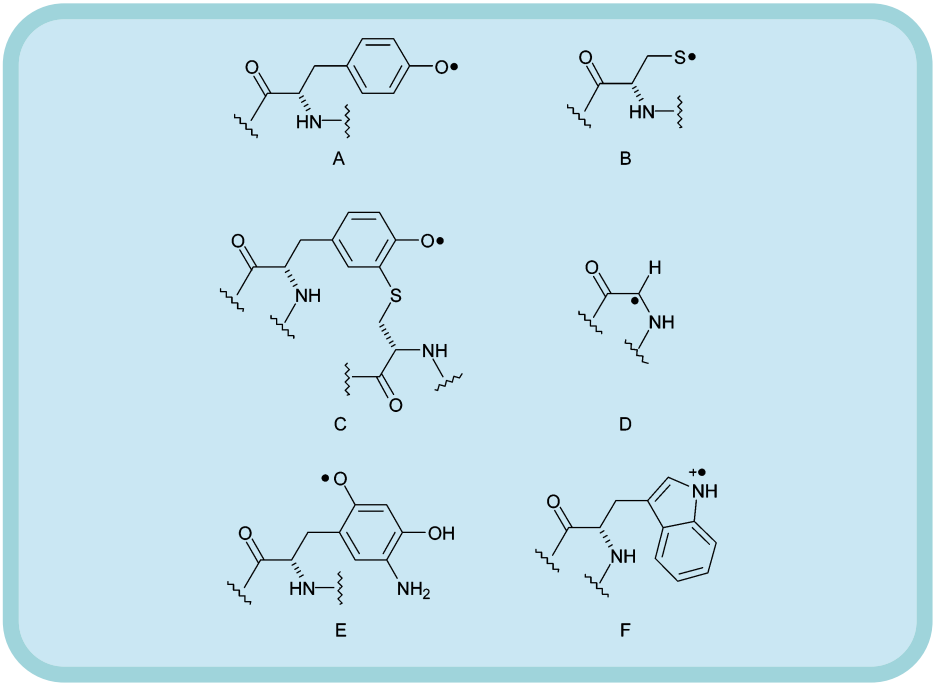

Fig. 1 Amino acid radicals involved in catalysis. A) Tyrosyl radicals are involved in the class I RNRs and in O_2 evolution in PSII; B) Thiyl radicals are involved in RNR and pyruvate formate lyase (PFL); C) The modified tyrosyl radical of galactose oxidase; D) Glycyl radicals involved in class III RNRs and PFL; E) A modified tyrosyl radical involved in plasma amine oxidase; F) Tryptophan cation radicals involved in cytochrome oxidase and assembly of the di-iron cluster in class I RNR.

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prevalent stable protein radicals are the tyrosyl or modified tyrosyl radicals (Fig. 1A and C) and the glycyl radical (Fig. 1D). Transient tyrosyl radicals (Fig. 1A and E), thiyl radicals (Fig. 1B) and tryptophan cation radicals (Fig. 1F) have also been observed. This commentary will specifically focus on the role of Y· in biochemical transformations.

Roles in metabolism

The Y· is essential to the catalytic activity of the class I RNRs, enzymes that play a central role in DNA replication and DNA repair.[1,4–6] RNRs catalyze the conversion of nucleotides to deoxynucleotides in all organisms and thus provide the monomeric precursors required for these processes (Fig. 2A). Y· radicals also play a central role in generation of O_2 by plants in photosystem II (PSII).[7] Two redox active tyrosines with very different properties (Y_Z and Y_D, the former is generated transiently and is kinetically competent in water oxidation, while the latter is stable) are part of a macromolecular manganese machine that in the presence of light can oxidize water to O_2 (Fig. 2B). A Y· has also been demonstrated to be a kinetically competent intermediate in prostaglandin synthase,[8] the rate-limiting step in prostaglandin biosynthesis and its intermediacy has been implicated in cytochrome oxidase,[9] an enzyme that plays a central role in respiration, reducing O_2 to water (Fig. 2).

Characterization of the Y·

In seminal experiments the 9 GHz electron paramagnetic resonance (EPR) signal observed in class I RNR from *E. coli* was assigned to a Y· (Fig. 3).[3] Using bacteria that were auxotrophic for tyrosine (require tyrosine for growth), allowed incorporation of specifically isotopically labeled tyrosines into all the bacterial proteins. Use of [β-^{2}H] -tyrosine in the growth media established that the doublet signal observed in the spectrum of RNR arose from hyperfine interactions associated with one of its two β-methylene protons. In the past decade high frequency (140, 285, 365 GHz) EPR (HF-EPR) and electron nuclear double resonance (HF-ENDOR) methods have provided a remarkable picture of the conformations and electronic structure of these radicals not accessible with low field methods or other spectroscopies.[10] These methods, in conjunction with rapid freeze quenching methods on the millisecond time scale or

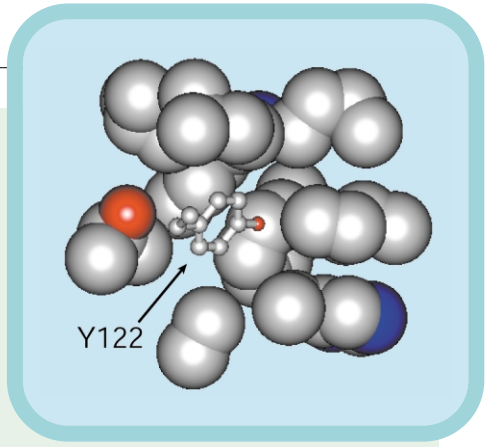

Fig. 3 The hydrophobic environment of the Y· of the *E. coli* class I RNR enhances its stability. The half life of this radical in the protein is several days and contrasts with the half life a Y· in solution which is sub-milliseconds.

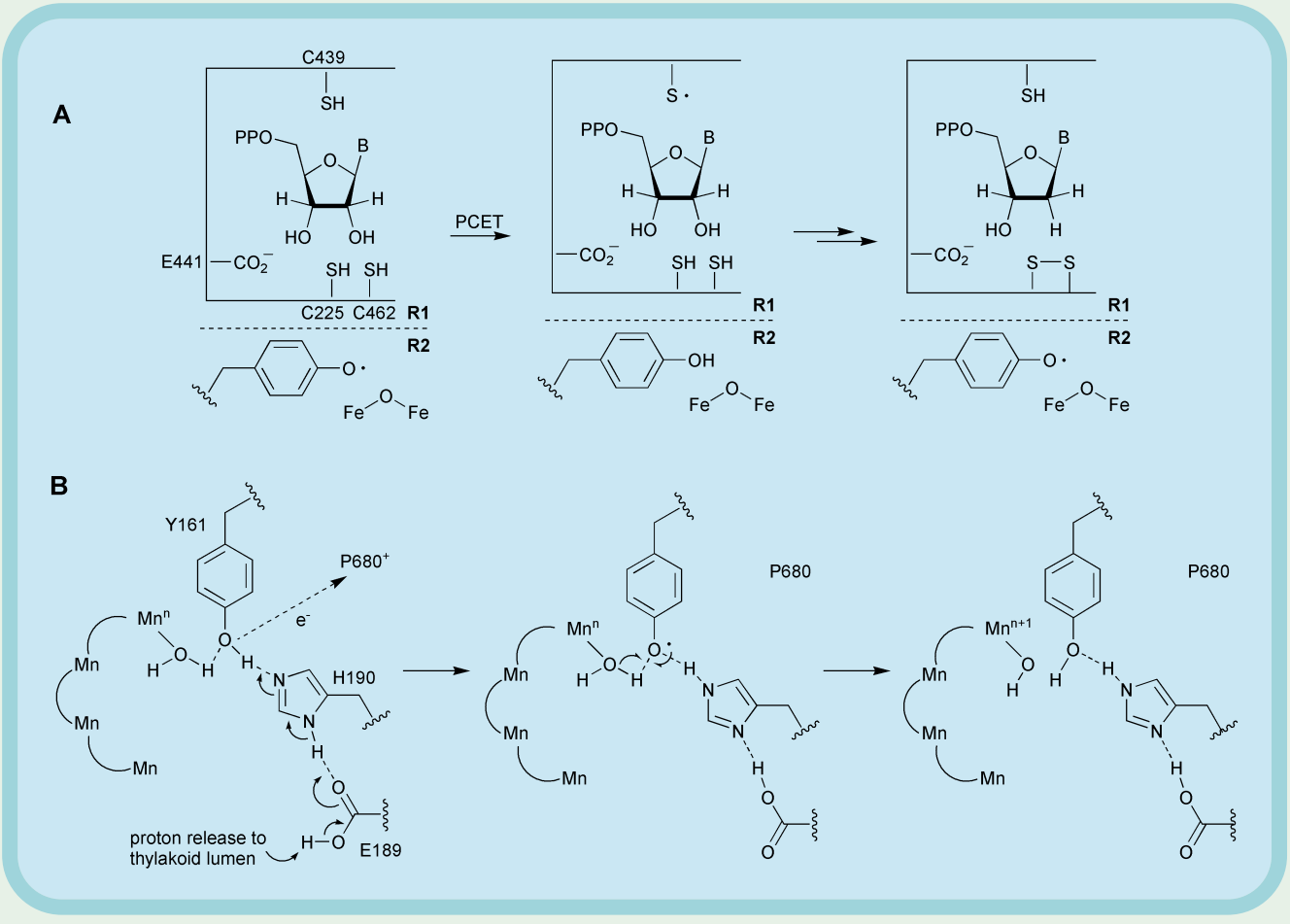

Fig. 2 A) Proposed mechanism of class I RNRs. The tyrosyl radical on subunit R2 generates a transient thiyl radical on subunit R1. Nucleotide reduction occurs and the tyrosyl radical on R2 is re-generated. B) Water splitting in PSII. The four oxidizing equivalents required to split water are generated stepwise by four consecutive photo-induced oxidations of the tetra-Mn cluster. Babcock's model in which Y161 (YZ) is oxidized by P680 to YZ· to abstract a hydrogen atom from a water bound to the Mn cluster. Only the first step of the multistep oxidation process is shown.

rapid-flow methods on the microsecond time scale, are also allowing a glimpse of the conformation and orientation of transient radical intermediates.[11]

Why Y·?

Several properties of tyrosine make this amino acid an excellent candidate for the transformations indicated in Fig. 2. Y· is a potent oxidant with the added bonus that its oxidation potential can be modulated by its protonation state, that in turn can be modulated by the protein environment.[12] The oxidation potentials are also within the range obtainable by common physiological oxidants.

The interrogation of the chemical role of Y· in RNRs and PSII mediated O_2 evolution is work in progress. At this stage it is difficult to say whether general paradigms will emerge. In the case of RNRs the Y· is located on one subunit of the protein (designated R2) and the active site where nucleotide reduction occurs is located on a second subunit (designated R1, Fig. 2A). The Y· in R2 is postulated to generate a transient thiyl radical in R1 which initiates the radical-dependent nucleotide reduction process. The Y· is thus functioning as a radical chain initiator. This initiation is postulated to occur over 35 Å and thought to involve long-range proton-coupled electron transfer through tyrosine and tryptophan radical intermediates.[13] The details of how the Y· is reduced and re-oxidized on conversion of each nucleotide to a deoxynucleotide remain a major focus of investigation and is unprecedented in biology.

PSII splits water to produce O_2 and uses the protons and electrons generated in this process for CO_2 reduction to carbohydrates. The role of light and the mechanism by which it generates sufficient oxidizing equivalents on a tetra-manganese cluster (Mn-cluster) to carry out this highly endergonic oxidation is also the topic of intense investigation.[14] Specifically, focus has recently turned to the transient Yz· and its role in water oxidation. Babcock made the novel proposal presently being investigated that this Y· may abstract hydrogen atoms from water and subsequent intermediates bound to the Mn cluster[7] (Fig. 2B). Thus, the chemical mechanism by which Y· radicals are involved in these and other systems, reduction by hydrogen atom abstraction or reduction by electron transfer coupled to proton transfer, remains an unsolved mystery in biology. Precedent for this chemistry is also receiving intense scrutiny in the chemical community.[15]

Generation of Y·

The methods of tyrosine oxidation to neutral radicals are also chemically diverse. The Y· in R2 of RNR and prostaglandin synthase are both oxidized by metal clusters. In the former case a non-heme di-ferrous cluster, O_2 and reductant are involved and in the prostaglandin synthase case, a ferric heme and alkyl peroxides are involved in generation of high valent iron species to affect these oxidations.[1] On the other hand PSII uses the energy of photons to create the oxidant.[14] The detailed mechanisms of biosynthesis of other amino acid radicals shown in Fig. 1 are areas of great interest. Here again, Nature is using reactive intermediates that if not highly constrained result in self-destruction.

Prognosis

Advances in technologies have established that, in contrast to what is taught in basic biochemistry text books, there are many more than 21 amino acids. One cannot pick up a journal without seeing yet another post-translational modification (amino acids within a protein are modified after the protein is synthesized on the ribosome) that affects and fine-tunes chemical reactivity. The modified amino acids shown in Fig. 1 are just another example of Nature's creativity in inventing new reactivities from old ones. In the case of RNR and PSII whoever would have imagined in their wildest dreams the solution to these chemical problems? New surprises in controlled radical chemistry remain on the horizon and many mysteries remain to be unraveled with powerful new technologies.

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