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Review

Vibrational spectroscopy to study the properties of redox-active tyrosines in photosystem II and other proteins

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Abstract

Tyrosine radicals play catalytic roles in essential metalloenzymes. Their properties—midpoint potential, stability. . .—or environment varies considerably from one enzyme to the other. To understand the origin of these properties, the redox tyrosines are studied by a number of spectroscopic techniques, including Fourier transform infrared (FTIR) and resonance Raman (RR) spectroscopy. An increasing number of vibrational data are reported for the (modified-) redox active tyrosines in ribonucleotide reductases, photosystem II, heme catalase and peroxidases, galactose and glyoxal oxidases, and cytochrome oxidase. The spectral markers for the tyrosinyl radicals have been recorded on models of (substituted) phenoxyl radicals, free or coordinated to metals. We review these vibrational data and present the correlations existing between the vibrational modes of the radicals and their properties and interactions formed with their environment: we present that the ν_{7a}(C–O) mode of the radical, observed both by RR and FTIR spectroscopy at 1480–1515 cm^{-1}, is a sensitive marker of the hydrogen bonding status of (substituted)-phenoxyl and $Tyr^{\bullet}$, while the ν_{8a}(C–C) mode may probe coordination of the $Tyr^{\bullet}$ to a metal. For photosystem II, the information obtained by light-induced FTIR difference spectroscopy for the two redox tyrosines Tyr_D and Tyr_Z and their hydrogen bonding partners is discussed in comparison with those obtained by other spectroscopic methods.

Keywords: Photosystem II; Tyrosinyl radical; Phenoxyl radical; Fourier transform infrared (FTIR) spectroscopy; Resonance Raman; Metallo-radical enzymes

1. Introduction

Among the free amino acid radicals identified in proteins, the redox couple tyrosyl radical/tyrosine was shown essential in many biologically important processes (see Ref. [1] for a review). It has been evidenced in key enzymes such as ribonucleotide reductase [2,3], which provides precursors of DNA synthesis, and photosystem II (PSII) [4,5]—the membrane protein complex where photosynthetic oxygen evolution takes place in plants, algae and cyanobacteria.

Ribonucleotide reductase: In class I ribonucleotide reductase (RNR), the redox active Tyr_{122} (amino acid numbering according to *E. coli* sequence) is located at 5–6 Å of the di-iron cluster on the R2 subunit (Fig. 1A) [6]. The $Tyr^{\bullet}$ is generated upon reduction of oxygen into water at the iron cluster (Ref. [7] and references therein). $Tyr^{\bullet}$ initiates the formation of a thiyl radical at the active site for ribonucleotide reduction on the R1 subunit. This thiyl radical, proposed to occur on Cys_{439}, in turn initiates catalysis on the nucleotide substrate [7–10].

Photosystem II: In PSII, two redox tyrosines, Tyr_D and Tyr_Z, have been evidenced, at homologous positions on the two polypeptides D2 and D1 forming the core of the membrane protein complex (reviewed in Refs. [11–13]). Light absorption at a chlorophyllic species so-called primary electron donor P_{680} induces a charge separation between P_{680} and an electron acceptor plastoquinone Q_A. Tyr_Z and/or Tyr_D reduce the highly oxidizing P_{680}^{+}, generating the Tyr radicals. Tyr_Z is located at ≈ 7 Å of the Mn_4-Ca^{2+} active center for oxygen evolution [14–18]. Tyr_Z is directly

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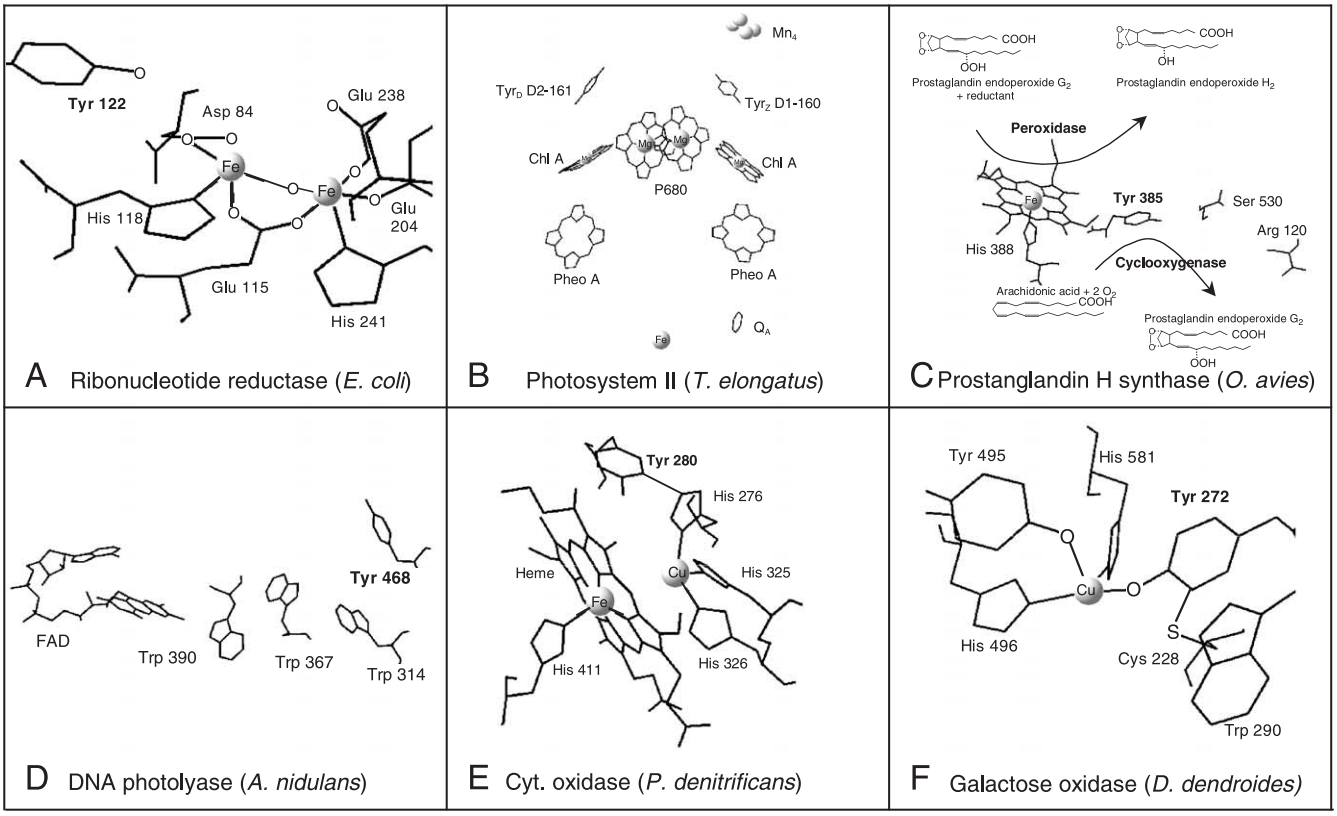

Fig. 1. Scheme of the Tyr• environment in (A) class I ribonucleotide reductase (modified from Ref. [6]), (B) photosystem II [16–18], (C) prostaglandin H synthase [151], (D) DNA photolyase of *A. nidulans* [152] with postulated Trp and Tyr residues involved in the electron-transfer reaction. (E) His-Tyr• at the Cu_B-Heme a3 of cytochrome *c* oxidase [35] and (F) cysteinyl-Tyr• at the active site of galactose oxidase or glyoxal oxidase [46].

involved in the physiological electron and proton transfer reactions associated with water oxidation into molecular oxygen (Fig. 1B, see below). Tyr_D is not essential for oxygen evolution. However, mutation of this strictly conserved redox tyrosine in the obligatory photoautotrophic *Thermosynechoccocus* species largely increases the growth generation time and tendency to revert [M. Sugiura et al. in preparation]. The interactions of Tyr_D with the Mn-Ca^{2+} cluster and P^{+}_{680} are reviewed in Ref. [19].

Stable or transient tyrosine radicals are also implicated in a variety of regulatory enzymes that are important in primary metabolism.

Prostaglandin synthases: Prostaglandin synthases are essential for the production of a variety of prostaglandins from arachidonic acid. This process implies both a cyclooxygenase reaction, which converts arachidonic acid to prostaglandin G_2, and the hydroxylation of prostaglandin G_2 into prostaglandin H_2 (Fig. 1C). This latter peroxidation reaction involves a highly oxidizing intermediate so-called compound I, an oxoferryl protoporphyrin IX radical cation ($FeIV{=}O\ PPIX^{+}$ •) [20,21] that generates Tyr• by intraprotein electron transfer. Tyr• in turn initiates the cyclooxygenase reaction by forming the arachidonate radical [22,23] which reacts with oxygen to yield prostaglandin G2. Crystallographic data revealed that the Tyr• would be located between the heme and arachidonate binding sites, well positioned to couple the two enzyme activities [24].

Catalases and peroxidases: More generally, for most catalases and peroxidases having a protoporphyrin prosthetic group at the active site, the highly oxidizing compound I or, alternately, an oxoferryl protoporphyrin IX and a protein-based radical species are generated by the two-electron oxidation of the protoporphyrin by alkhyl- or hydrogen-peroxides [25]. A Trp• intermediate relevant for substrate oxidation was observed for cytochrome *c* peroxidase [26], while formation of an oxoferryl protoporphyrin IX-Tyr• intermediate was evidenced in beef liver catalase [27], turnip and cytochrome *c* peroxidases [28,29] and catalase-peroxidases from *Mycobacter tuberculosis* and *Synechocystis* [30,31].

DNA photolyase and cryptochrome-1: Finally, an electron transfer from a tyrosine residue to a tryptophane radical was detected in the DNA photolyase of *Anacystis nidulans* [32] (Fig. 1D), while tryptophane radicals only were detected in the same enzyme of *E. coli* [33]. DNA photolyases are flavoproteins that catalyze UV-induced repair of major UV-damaged DNA lesions by reduction of pyrimidine dimers. Recently, a transient Tyr• was also detected in the blue light photoreceptor cryptochrome-1 from *Arabidopsis thaliana* [34] which shows large sequence homology with DNA-photolyase.