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Review

Reaction dynamics and proton coupled electron transfer: Studies of tyrosine-based charge transfer in natural and biomimetic systems ☆

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ABSTRACT

In bioenergetic reactions, electrons are transferred long distances via a hopping mechanism. In photosynthesis and DNA synthesis, the aromatic amino acid residue, tyrosine, functions as an intermediate that is transiently oxidized and reduced during long distance electron transfer. At physiological pH values, oxidation of tyrosine is associated with a deprotonation of the phenolic oxygen, giving rise to a proton coupled electron transfer (PCET) reaction. Tyrosine-based PCET reactions are important in photosystem II, which carries out the light-induced oxidation of water, and in ribonucleotide reductase, which reduces ribonucleotides to form deoxynucleotides. Photosystem II contains two redox-active tyrosines, YD (Y160 in the D2 polypeptide) and YZ (Y161 in the D1 polypeptide). YD forms a light-induced stable radical, while YZ functions as an essential charge relay, oxidizing the catalytic Mn_4CaO_5 cluster on each of four photo-oxidation reactions. In *Escherichia coli* class 1a RNR, the β2 subunit contains the radical initiator, Y122O•, which is reversibly reduced and oxidized in long range electron transfer with the α2 subunit. In the isolated *E. coli* β2 subunit, Y122O• is a stable radical, but Y122O• is activated for rapid PCET in an α2β2 substrate/effector complex. Recent results concerning the structure and function of YD, YZ, and Y122 are reviewed here. Comparison is made to recent results derived from bioengineered proteins and biomimetic compounds, in which tyrosine-based charge transfer mechanisms have been investigated. This article is part of a Special Issue entitled: Vibrational spectroscopies and bioenergetic systems.

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1. Introduction

In photosynthesis and respiration, electrons must be transferred long distances across a membrane. Electron transfer (ET) reactions are mediated by tunneling with rates that decrease exponentially with distance [1]. To accelerate bioenergetic ET reactions and make them biologically useful, redox-active cofactors are embedded into the protein matrix [2]. These redox-active cofactors are transiently oxidized and reduced and define a radical transport pathway. The aromatic amino acid, tyrosine, serves as such a redox-active cofactor in photosynthesis and DNA synthesis (reviewed in ref. [3]). Tyrosine charge transfer has also been proposed to play important roles in other metabolic processes, including galactose oxidation [4], prostaglandin synthesis [5], fatty acid oxidation [6], peroxide disproportionation [7], and oxygen reduction in cytochrome c oxidase [8]. While tyrosyl radicals, generated by photolysis in solution, decay in the microsecond time regime [9], proteins have developed ways to control radical reactivity and extend radical lifetimes. The intermediate tyrosyl radical can be detected using spectroscopic techniques, such as time-resolved optical, electron paramagnetic resonance (EPR), and vibrational spectroscopies (visible Raman, UV resonance Raman (UVRR), and reaction-induced FT-IR (RIFT-IR)). These techniques have yielded insight into the functional roles of redox-active tyrosines.

When tyrosine is oxidized, a neutral tyrosyl radical is formed [10]. It has been proposed [3] that deprotonation of the phenolic oxygen, accompanying radical formation, is important in controlling the kinetics and thermodynamics of ET under physiological conditions. Fig. 1 illustrates a reaction, in which oxidation of tyrosine is accompanied with protonation of an imidazole side group of histidine. This reaction is an example of a proton coupled electron transfer (PCET) reaction. Because proton transfer (PT) can occur only over short distances, the position, structure, and pK_a of the proton-accepting group are potentially critical factors in controlling radical transfer (for example, see

Abbreviations: Chl, chlorophyll; EPR, electron paramagnetic resonance; ET, electron transfer; NMR, nuclear magnetic resonance; OEC, oxygen evolving complex; PCET, proton coupled electron transfer; Pheo, pheophytin; PT, proton transfer; PSII, photosystem II; Q_A and Q_B, plastoquinone acceptors in PSII; RIFT-IR, reaction-induced FT-IR; RNR, ribonucleotide reductase; SIE, solvent isotope effect; UNA, unnatural amino acid; UVRR, ultraviolet resonance Raman, Y122, tyrosine 122 in the β2 subunit of RNR; YD, tyrosine 160 in the D2 polypeptide of PSII; YZ, tyrosine 161 in the D1 polypeptide of PSII; υ_{as}, asymmetric stretch, υ_s, symmetric stretch

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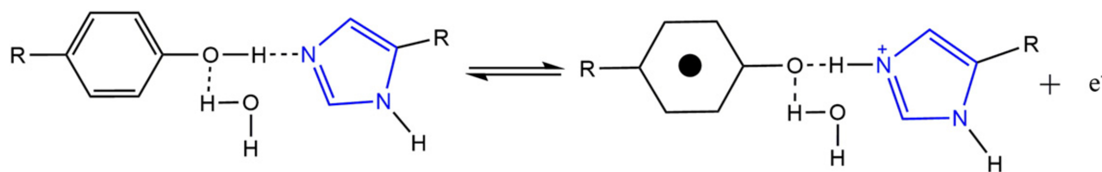

Fig. 1. Schematic diagram illustrating a PCET reaction between tyrosine and a hydrogen-bonded histidine.

discussion in [11]). The mechanisms of tyrosine-based ET and PCET reactions in DNA synthesis, photosynthesis, bioengineered proteins, and biomimetic compounds have been the subject of great interest, and some recent findings, relevant to tyrosine-based charge transfer, are reviewed here.

2. Ribonucleotide reductase

The prototypical example of a tyrosine-based charge relay occurs in ribonucleotide reductase (RNR). In all RNRs, the reduction of ribonucleotides to deoxyribonucleotides proceeds through a free radical mechanism [12]. The reaction is initiated by H atom abstraction at the ribose 3′-carbon using an active site, transient cysteine radical [13]. There are three classes of RNRs, grouped according to the redox-active cofactor that is used as the radical initiator [14,15]. Class Ia RNRs use a tyrosyl radical (Y122O•)-diferric cofactor to generate the cysteine radical. Class Ia RNRs are found in humans, viruses, and some bacteria, including *Escherichia coli*, and are composed of α2 (formerly R1) and β2 (formerly R2) subunits (reviewed in [14]).

The binding and reduction of substrate occur in the ~170 kDa α2 subunit (Fig. 2). The C439 radical generates a 3′ substrate radical by H atom abstraction from the substrate, NDP [14]. To regulate activity, class Ia α2 contains two effector sites, one termed the specificity site and the other termed the overall activity site. When bound to the activity site, dATP is a reversible inhibitor [16]. In *E. coli*, dATP binding to the activity site has been shown to form inactive, α4β4 oligomers [17–19]. Effectors, such as ATP, bind to the specificity site and promote CDP or UDP reduction [20]. Further, binding at the specificity site stimulates interactions between α2 and β2 [17–19]. Thus, RNR is a dynamic molecule that is under exquisite allosteric and oligomeric control.

In the 87 kDa *E. coli* β2 subunit, the tyrosyl radical, Y122O•, oxidizes C439 via a reversible, long distance PCET process (Fig. 2). The Y122 radical is required for activity [21] and is generated by oxygen-requiring redox reactions at a diiron cluster [22–24]. In the isolated β2 subunit, Y122O• is stable for days [25]. However, formation of an α2β2 substrate/effector complex activates Y122O• for rapid PCET (reviewed in [26]). While the chemistry of nucleotide reduction has been shown to be ~100 s^{-1} [27], the overall activity or RNR is slower and gated by structural changes (2–10 s^{-1}) [28].

Radical propagation between Y122 and C439 occurs over 35 Å between the two subunits [29,30] via a conserved pathway of aromatic side chains. The pathway involves a reversible, proton coupled electron transfer (PCET) process (β2: Y122O• ⇋ [W48] ⇋ Y356 ⇋ α2: Y731 ⇋ Y730 ⇋ C439) (reviewed in [26]). The roles of residues in the PCET pathway were elucidated using site-directed mutagenesis [31–33] and site-specific incorporation of unnatural amino acids (UNAs) [27,34–40]. Substitution of tyrosines with aminotyrosine or fluorotyrosine trapped metastable intermediates in radical transfer (reviewed in [26]).

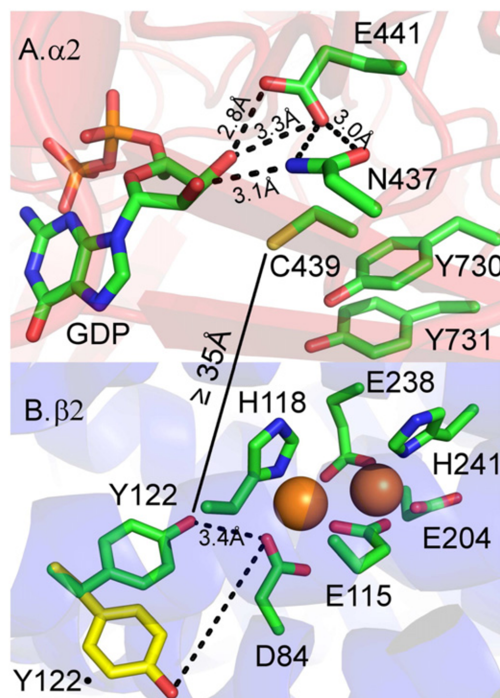

Fig. 2. Structures of the α2 and β2 subunits of RNR. (A) Active site residues in the α2 subunit at 3.2 Å (PDB 4R1R) and containing the substrate, GDP; (B) Y122OH-differric cluster in β2 at 1.9 Å (PDB 1MXR). The solid line is the distance [29,108] between β2 Y122 and α2 C439. The proposed conformational change at Y122O• is superimposed in part B in yellow, and is described as a singlet A (backbone/ring dihedral angles: 173°/99°) to a radical B (−69°/80°) conformational change in a YT dipeptide [51]. Iron atoms are shown as orange spheres. Y356 is located in a disordered region of the β2 structure and is not shown.

3. The Y122O• radical initiator in RNR

As mentioned above, in class 1a RNR, Y122O• functions as a radical initiator in a PCET pathway that involves multiple, aromatic amino acids. The substitution of UNAs into this conserved PCET pathway (Y122 β2, Y356 β2, Y731 α2, Y730) has shown that forward ET is slightly uphill in energy and mostly likely driven by the irreversible release of water from the substrate (reviewed in [26]). At physiological pH values, tyrosine residues are expected to deprotonate when oxidized [10]. Site-specific mutagenesis has identified the pK_as of Y122, Y356, Y731, and Y730 and has shown that these residues are protonated in the pH regime in which RNR is active (pH 6–8) [41]. Therefore, PT must normally be associated with ET both in the β2 and in the α2 subunits. In the β2 subunit, experimental data suggests that PT and ET are orthogonal, meaning that the proton and electron are transferred to different acceptors. In the α2 subunit, evidence suggests that ET and PT are collinear, with the proton and electron transferring to the same acceptor (reviewed in [26,42]).

The kinetics of substrate reduction was defined in a nitrotyrosine derivative, NO_2Y122OH, in which the conformational gate is uncoupled from the chemistry of substrate reduction [27]. This NO_2Y122OH mutant conducts one enzymatic turnover, but is inactivated for multiple turnovers. However, substitution of NO_2Y at other tyrosines in the PCET pathway dramatically decreased the activity of RNR [41]. Significantly, X-ray structures of the NO_2Y α2 mutants [41] and the NO_2Y122OH mutant [27] revealed no significant structural changes relative to wildtype.

Although the midpoint potential of NO_2Y122OH is expected to be increased relative to Y122OH (~200 mV), the NO_2Y122O• species was generated by activation at the iron cluster, forming a metastable radical (~40 s half-life, 1.2 radical per β2). After mixing with substrate and effector and the α2 subunit, the NO_2Y122O• mutant generated 0.6 equivalent of dCDP and ~0.6 equivalent of a new radical on the pathway [27].

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