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Review

Structure and interactions of amino acid radicals in class I ribonucleotide reductase studied by ENDOR and high-field EPR spectroscopy

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Abstract

This short review compiles high-field electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) studies on different intermediate amino acid radicals, which emerge in wild-type and mutant class I ribonucleotide reductase (RNR) both in the reaction of protein subunit R2 with molecular oxygen, which generates the essential tyrosyl radical, and in the catalytic reaction, which involves a radical transfer between subunits R2 and R1. Recent examples are presented, how different amino acid radicals (tyrosyl, tryptophan, and different cysteine-based radicals) were identified, assigned to a specific residue, and their interactions, in particular hydrogen bonding, were investigated using high-field EPR and ENDOR spectroscopy. Thereby, unexpected diiron-radical centers, which emerge in mutants of R2 with changed iron coordination, and an important catalytic cysteine-based intermediate in the substrate turnover reaction in R1 were identified and characterized. Experiments on the essential tyrosyl radical in R2 single crystals revealed the so far unknown conformational changes induced by formation of the radical. Interesting structural differences between the tyrosyl radicals of class Ia and Ib enzymes were revealed. Recently accurate distances between the tyrosyl radicals in the protein dimer R2 could be determined using pulsed electron–electron double resonance (PELDOR), providing a new tool for docking studies of protein subunits. These studies show that high-field EPR and ENDOR are important tools for the identification and investigation of radical intermediates, which contributed significantly to the current understanding of the reaction mechanism of class I RNR.

Keywords: Ribonucleotide reductase; High-field EPR; ENDOR; Tyrosyl; Tryptophan; Cysteine radical

1. Introduction

Ribonucleotide reductase (RNR) is a long known and probably the best-investigated radical enzyme. It catalyses the reduction of ribonucleotides to deoxyribonucleotides (Fig. 1), which is the rate-limiting step of DNA synthesis [1–5]. Therefore, RNR is an important target for cell growth control, and several RNR inhibitors are being used, or have been proposed, as drugs for chemotherapeutic treatment of cancer and virus infections based on radical scavenging, substrate analogues, or on peptidomimetic inhibitors [4–8]. Three main classes of RNRs have been described, classified according to the radical generator driving the catalytic reaction [4,8–13]. Class I enzymes produce a stable tyrosyl radical on one protein subunit in a reaction of a dinuclear iron center with molecular oxygen [1–5]. The Class II enzymes use the cofactor cobalamin for radical generation [4,12–14]. A thiyl radical, strongly coupled to the cobalt ion of the cobalamin, was reported to be the active radical, which drives substrate turnover in class II RNR [12,13]. Class III enzymes are strictly anaerobic and form a stable glycyl radical with the help of an iron–sulfur protein and *S*-adenosyl methionine [9–11,15]. Two landmarks for the

Abbreviations: EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; RNR, ribonucleotide reductase; R2, subunit of class I RNR, which carries the diiron site and the active tyrosyl radical; R1, subunit of class I RNR, which binds the substrate; hf, hyperfine; g, electronic g-tensor with principal values g_x, g_y, and g_z; Y122$^\bullet$, Y177$^\bullet$, W111$^\bullet$, W177$^\bullet$, tyrosyl and tryptophan radicals in wild-type and mutant R2 of *E. coli* and mouse

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investigation of the last two classes were the X-ray structure analysis for the anaerobic class III enzyme in 1999 by Logan et al. [15] and recently, in 2002, also for the class II enzyme by Sintchak et al. [14].

Class I enzymes are found in practically all eukaryotic organisms, from yeast and algae to plants and mammals, and some prokaryotes and viruses also express this type. Class I RNR of *E. coli* was mostly used for studying structure/function or/reactivity relationship by investigating the wild-type and various mutants (for reviews, see Refs. [1–5,8]). It consists of two homodimeric proteins, R1 and R2, see Fig. 1 [1–5]. While the substrate turnover reaction is performed in R1, the role of protein R2 is to harbor in the active state a tyrosyl radical, Y122$^{\bullet}$ (*E. coli* numbering), which is needed for starting the catalytic reaction in R1 (see Fig. 1). The tyrosyl radical is located close to a diferric iron center, which couples antiferromagnetically to form an $S=0$ ground state. The X-ray structures have been determined separately for R2 [16] and also for R1 with substrate and one affector bound [17], see Fig. 1.

Catalytic models have been proposed based on these structural data. Catalytic activity requires a holoenzyme complex R1–R2, the structure of which is yet not known in detail. The catalytic reaction in R1 is believed to involve a coupled electron/proton (H$^{\bullet}$ radical) transfer via a conserved hydrogen-bonded pathway from the tyrosyl radical Y122$^{\bullet}$ in protein R2 to cysteine C439 in R1 (*E. coli* numbering). In R2, the pathway is made up of the iron ligands D84 and H118, and continues via D237 and W48, which is located close to the protein surface. In R1, two tyrosines Y730 and Y731 and cysteine C439, which is at the substrate binding site, are involved in the pathway, see Fig. 2 and Refs. [1–5,8,18–21]. The substrate turnover reaction in R1 is proposed to be initiated by a thiyl radical on C439

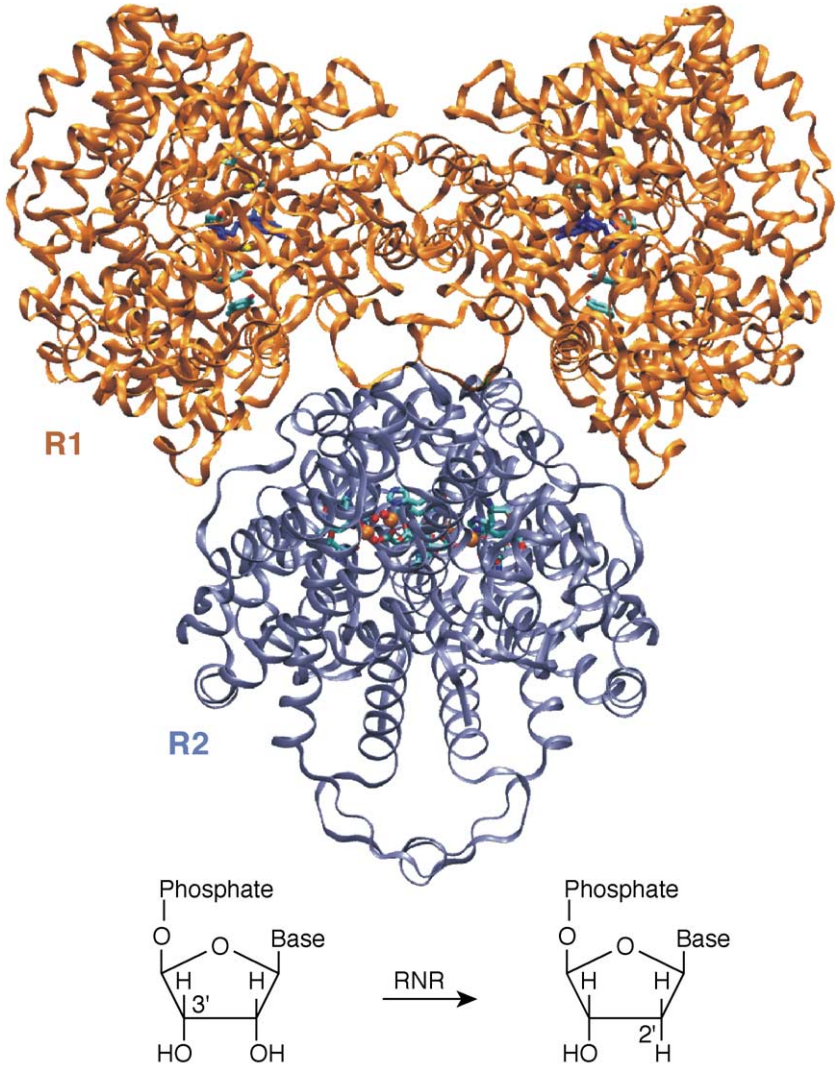

Fig. 1. X-ray structure of ribonucleotide reductase subunits R1 (gold) with bound substrate GDP and oxidized met R2 (blue) from *E. coli* [16,17,49]. The two Fe^{III} (yellow) with bridging oxygen and terminal water ligands (red), histidine ligands, and the tyrosine Y122 (cyan) in R2 and GDP (blue) in R1 are indicated. Relative arrangement of both subunits in analogy to Sjöberg [1]. Bottom: Reduction of a nucleotide to a deoxynucleotide.