Coordination Chemistry Reviews 257 (2013) 3–26

Contents lists available at SciVerse ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Ribonucleotide reductase class I with different radical generating clusters

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Contents

ARTICLE INFO

Article history:
Received 29 February 2012
Received in revised form 16 May 2012
Accepted 19 May 2012
Available online 23 June 2012

ABSTRACT

Ribonucleotide reductase (RNR) catalyzes the rate limiting step in DNA synthesis where ribonucleotides are reduced to their corresponding deoxyribonucleotides. They are formed through a radical-induced reduction of ribonucleotides. Three classes of RNR generate the catalytically active site thiyl radical using different co-factors: a tyrosyl-radical in most cases (class I), homolytic cleavage of

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0010-8545/$ – see front matter
http://dx.doi.org/10.1016/j.ccr.2012.05.021

Keywords:
Ribonucleotide reductase
R2 subunit
Di-iron metal cluster
Di-manganese metal cluster
Electron paramagnetic resonance (EPR)
Resonance Raman (rRaman)
Circular dichroism/magnetic circular dichroism (CD/MCD)
Density functional theory (DFT)
SQUID magnetic measurement

deoxyadenosyl-cobalamin (class II), or a glycyl-radical (class III), respectively. Class I RNR has a larger subunit R1/R1E containing the active site and a smaller subunit R2/R2F with (the thiyl-generating power from) a tyrosyl radical or an oxidized iron-manganese cluster and is reviewed herein. Class I is divided into subclasses, Ia (tyrosyl-radical and di-iron-oxygen cluster), Ib (tyrosyl-radical and di-manganese-oxygen cluster) and Ic (an iron-manganese cluster). Presented here is an overview of recent developments in the understanding of class I RNR: metal-ion cluster identities, novel 3D structures, magnetic-optical properties, and reaction mechanisms. It became clear in the last years that the primitive bacterial RNR sources can utilize different metal-ion clusters to fulfil function. Within class Ia that includes members from eukaryotes (mammalians, fish) and some viruses species, the presence of hydrogen bonding interactions from water at different distances with the tyrosyl-radical site can occur. This demonstrates a large versatility in the mechanism to form the thiyl radical.

1. Introduction

One of the essential processes for sustaining life in all organisms is the availability of a balanced pool of DNA building blocks for processes such as cell division and DNA damage repair. Ribonucleotide reductase (RNR) is the enzymatic machine that maintains this pool of DNA precursors together with the so-called salvage pathways. RNR catalyzes reduction of the four main ribonucleotides to their corresponding deoxyribonucleotide diphosphates or deoxyribonucleotide triphosphates depending on the three classes of RNR in Scheme 1, interestingly some viruses have their own RNR [1–8]. The RNR system represents both the initial and the rate limiting step in the DNA-synthesis, as this process is allosterically regulated, especially in higher organisms. Subtle differences exist in class I RNR, depending on the living organisms, such as the presence of different hydrogen bonds to the tyrosyl radical in mammalian RNR, which are absent in less evolved species. RNR can use of different metal cofactors and can employ of different sources of reducing equivalents to reduce a di-sulfide to active cysteines.

RNRs activity is highly transcriptionally regulated and cell phase dependent. The first RNR protein was discovered in the 60s by Reichard and co-workers [2–4,9] in *Escherichia coli*. They noted an unprecedented reaction involving ribonucleotides, where a carbon bound OH-group could be directly replaced by hydrogen atom [2–4,7,9]. A few years later, the first RNR operon was cloned and sequenced [2–4,9], followed by successful cloning of genes from mouse [5,6] and yeast [5,6]. Since then, the growing numbers of genomic DNA sequences responsible for functional coding of RNRs have been revealed in various organisms, and today all known RNR protein sequences are collected in a database, the RNRdb (Ribonucleotide Reductase database). The aim of this archive is to provide a knowledge-transfer resource for exploration of RNR diversity and distribution in Nature (http://rnrdb.molbio.su.se) [8]. RNR is vital to rapidly dividing cells and after DNA-damage, e.g. thus highly relevant to cancer and bacterial or viral infections. During the last years novel and essential developments have occurred in the RNR field, e.g. (i) Phase Ib Clinical Trials for a cancer treatment using siRNA against RNR R2 (called CALAA-01 (http://www.calandopharma.com/technology/rondel/in-the-clinic/)), (ii) new 3D structures of both the novel di-manganese forms of RNR, the flavoprotein–RNR complex as well as other new class Ib RNR proteins NrdI and NrdH, (iii) the complex metal-ion cluster forming system start to be unraveled, and (iv) structural studies of human and yeast RNR have advanced (published in *Nature* [10], *Science* [11,12], *Angew. Chem. Int. Ed.* [13], *Nature Struct. Mol. Biol.* [14,15]).

RNRs are structurally and functionally complex molecular machines (Scheme 1). They utilize a free radical mechanism to exchange the hydroxyl group on the 2′-position of the ribose ring with a hydrogen atom [5–7] and all known RNRs contain two distinct functional components, a radical generator and a reductase substrate binding site. They share a common catalytic mechanism, with the activation of the ribonucleotide though abstraction of the 3′-hydrogen atom of the ribose by a transient thiyl radical in the enzyme active site in the large subunit. However, they differ in the chemical nature of the radical generators and depend on protein source. RNR enzymes have been grouped into three different classes (I, II, and III), based on differences in metal cofactors, their interaction with molecular oxygen, genetics and overall protein organization [2,6,7,16,17]. The three main classes reveal large differences in the mechanisms for the thiyl radical generation [1]. Most of the class I enzymes contain a relatively stable tyrosyl radical, located a few Ångströms from the di-metal oxygen cluster. Class II uses a radical on the cobalt containing cobalamin cofactor (vitamin B_{12}), and class III forms a stable glycyl radical with the aid of an iron–sulfur cluster coupled to S-adenosylmethionine (SAM or AdoMet). The large similarity of the catalytic domains among the three classes of RNR suggests the occurrence of rather similar reaction mechanisms (Fig. 1). The overall protein activities are regulated by binding of ATP/dATP to the large subunit. ATP is a general activator, while dATP acts as a feedback inhibitor, and the most RNR enzymes are often strictly allosterically regulated [5–9]. Class I RNRs share a common four-α-helix bundle housing a stable dimetal cluster containing iron or manganese as cofactors. Oxo, hydroxo, or aqua bridges are present between the two metal ions, depending on the protein isoform and/or oxidation state of the di-metalion-oxygen cluster [16].

The 3D structures, all show an active site cysteine that is organized in a similar fashion. These sites are placed in the center of a 10-stranded α/β-barrel, with the thiyl radical at the tip of a finger loop. Several bacteria can express two or three different types of RNRs, in response to their growing environment, but in higher organisms only class I has been found [1,5–9,16,17].

This review collects the current knowledge of the mechanism of oxygen activation in class I RNR, the presence and biological relevance of diverse metallo cofactors and our recent observations on RNR R2 proteins obtained from human and mouse (p53R2), the fish crucian carp, *Bacillus cereus* and *Epstein Barr* virus. The survey highlights how spectroscopic methods have been utilized

Abbreviations: CD, circular dichroism; dNDP, deoxyribonucleotidediphosphates; dNTP, deoxyribonucleotidetriphosphates; EBV, Epstein Barr virus; EPR, electron paramagnetic resonance; ENDOR, electron-nuclear double resonance; EXAFS, extended X-ray absorption fine structure; HF-EPR, high field EPR; HSV, herplex simplex virus; IR, infrared; MCD, magnetic circular dichroism; MMOH, hydroxylase component of methane monooxygenase; Mn-Cat, manganese catalase; mtDNA, mitochondrial DNA; NDP, ribonucleotidediphosphates; $Nrdl_{hq}$, fully reduced NrdI; NTP, ribonucleotidetriphosphates; PCET, proton-coupled electron transfer; PELDOR, pulsed electron–electron double resonance; R1, large subunit of class Ia RNR; R2, small subunit of class Ia RNR; R1E, large subunit of class Ib RNR; R2F, small subunit of class Ib RNR; RFQ, rapid freeze quench; RNR, ribonucleotide reductase; rRaman, resonance Raman; SAM, s-adenosylmethionine; SQUID, superconducting quantum interference device; VTVH, variable-temperature variable-field magnetic circular dichroism; ZFS, zero-field splitting.