Structure and Reaction Mechanism of Ribonucleotide Reductase

Jiangyun Wang Literature Seminar November 30, 1999

Ribonucleotide Reductases (RNR) catalyze the conversion of nucleotides into deoxynucleotides. The nucleotides are the building block of DNA, the source of genetic information found in all living cells (Fig. 1). Four classes of RNRs have been identified based on their inorganic cofactors, Class I contains a diferric confactor, Class II contains a Vitamin B_{12} cofactor, class III has an Fe_4S_4 confactor while Type IV contains a di manganese confactor.[1] Interestingly, all RNRs use protein radicals to activate the ribonuceotide substrate.[2]

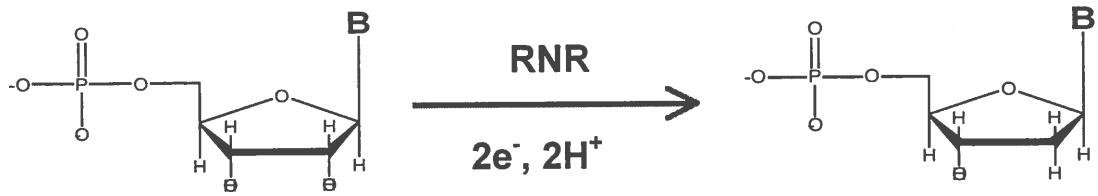

Figure 1

The class I RNR has received most attention because of its common occurrence in eukaryotes and E. coli.[3] The molecular organization of this protein is an enzyme where the two homodimers are denoted R1 and R2.[4] The R1 protein contains the allosteric nucleotide binding site and active site, whereas each subunit of the R2 protein contains a radical located on Tyr122 close to the dinuclear iron center.[5]

Organic radicals are highly reactive and cannot be free in cells. In this enzyme, the radical reaction is not initiated until the substrate is bound to the active site of the protein. The Tyr122 (R2) radical is buried 10Å inside the R2 subunit and is protected by the hydrophobic core. When the substrate binds R1, an electron is then transferred from Cys439 (R1) to Tyr122 (R2); Cys439 being the active residue in the reaction. After the substrate leaves the binding pocket, the electron is transferred back to Cys439, the Cys439 radical is eliminated, and Tyr122 radical is recovered, the next cycle then begins. In short, Tyr122 (R2) is an on/off button and Cys439 (R1) is a light bulb.[5,6,7]

The class I RNR is aerobic; it needs oxygen to be activated. Modern spectroscopic methods were used extensively to delineate the reaction mechanism. The active enzyme (**P**) contains a tyrosyl radical, which has an absorption at 412nm, stopped-flow UV-V is was used to measure the rate of the reaction.[6] Structural information of Intermediate **X** was also obtained using other spectroscopic methods. Q-band EPR shows an anisotropic signal around g=2,[8] which implies that it contains an unpaired electron on iron atoms with S=1/2 spin state.[8] ^{57}Fe ENDOR shows that the Fe(A) site is isotropic while Fe(B) site is anisotropic, suggesting a mixed-valent Fe(III)-Fe(IV) cluster,[8] that is further confirmed by magnetic Mössbauer.[8] From EXAFS the distance from the two iron atoms is found to be 2.5Å,[11] suggesting two or three μ-Oxo bridges. Four different possible structures were then proposed, and further

identified by ^{17}O ENDOR.[9] In addition, CD and MCD provided information about the electronic structure of the metal center,[12] and model studies by Lippard and Coworkers confirmed the structure and mechanism of the active site.[13]

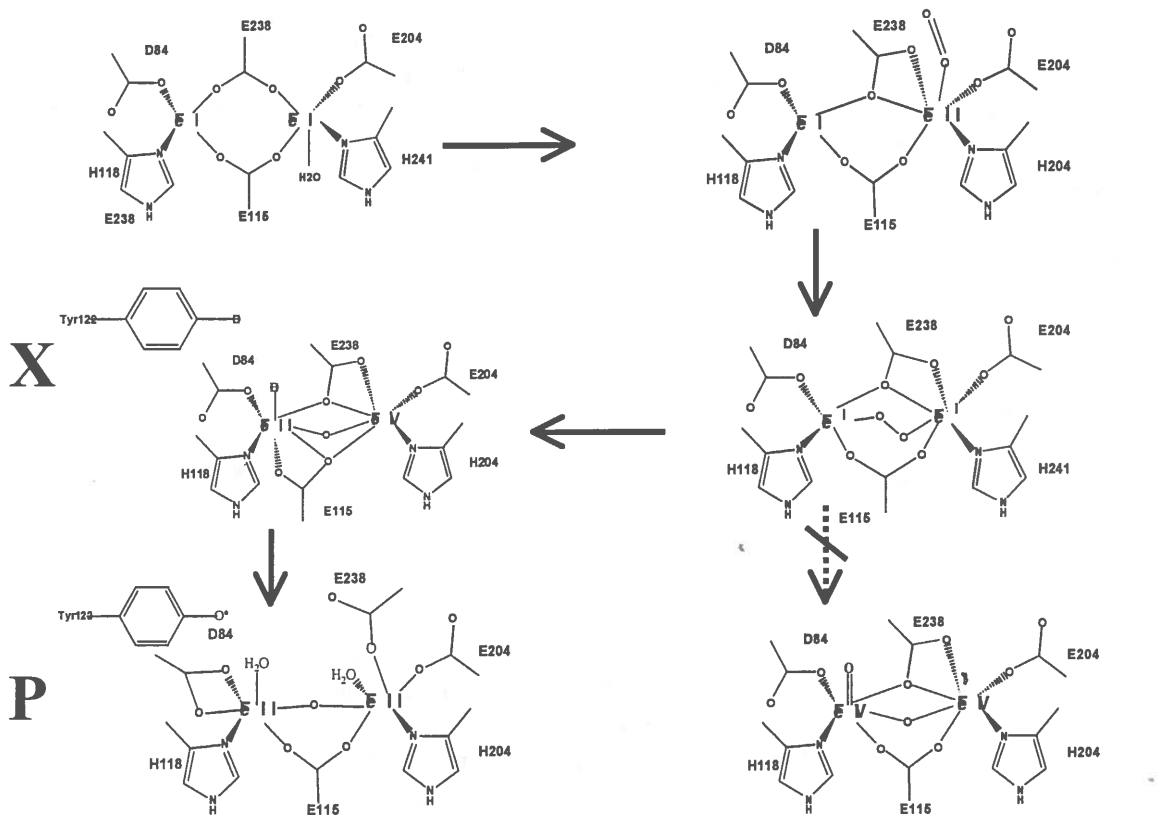

Figure 2

As a ubiquitous enzyme in all living cells, RNR has been extensively studied since it was first discovered in early 1950s. The reaction mechanism of Type I has been relatively clear, but still a lot of work need to be done to delineate the structure and mechanism of all the other types of RNRs.

References

1. Stubbe, J. and Van der Donk. W. A. "Protein Radicals in Enzyme Catalysis," *Chem. Rev.* **1998,** 705-762.

2. Licht, S.; Gerfen, G. J.; Stubbe, J. "Thiyl Radicals in Ribonucleotide Reductase," *Science* **1996**, 271, 477-481.

3. Jordan, A.; Reichard, P. *Annu. Rev. Biochem.* "Ribonucleotide Reductase," **1998,** 67,71-98.

4. Uhlin, U. and Eklund, H. "Structure of Ribonucleotide Reductase Protein R1," *Nature* **1994**, 370, 533-539.

5. Norlund, P.; Sj➢berg, B.; Eklund, H. "Three-dimensional Structure Of the Free Radical Protein Of Ribonucleotide Reductase," *Nature* **1990**, 345, 593-598.

6. Bollinger, J. M.; Edmondson, D. E.; Huynh, B. H.; Filley, J.; Norton, J. R.; Stubbe, J. "Mechanism Of Assembly Of the Tyrosyl Radica-Dinulcear Iron Cluster Cofactor Of Ribonucleotide Reductase," *Science* **1991,** 253, 292-298.

7. Reichard, P.; Ehrenberg. A. "Ribonucleotide Reductase— A Radical Enzyme," *Science* **1983**, 221, 514-519.

8. Sturgeon, B. E.; Burdi, D.; Chen, Shuxian; Huynh, B-H.; Edmonson, D. E.; Stubbe, J.; Hoffman, B. M. "Reconsideration Of X, the Diiron Intermediate Formed During Cofactor Assembly In E. Coli Ribonucleotide Reductase," *J. Am. Chem. Soc.* **1996**, 118, 7551-7557.

9. Burdi, D.; Willems, J-P.; Riggs-Gelasco, P.; Anthioline, W. E.; Stubbe, J.; Hoffman, B. M. "The Core Structure Of X Generated In the Assembly Of the Diiron Cluster Of Ribonucleotide Reductase: ^{17}O and H_2 ^{17}O ENDOR," *J. Am. Chem. Soc.* **1998**, 120, 12910-12919.

10. Bollinger, J. M.; Tong, W. H.; Ravi, N.; Huynh, B. H.; Edmondson, D. E.; Stubbe, J. "Mechanism Of Assembly Of Tyrosyl Radical-Diiron(III) Cofactor Of E. Coli Ribonucleotide Reductase. 3. Kinetics Of the Limiting Fe^{2+} Reaction By Optical, EPR, and Mössbauer Spectroscopies," *J. Am. Chem. Soc.* **1994**, 116, 8024-8032.

11. Riggs-Gelasco, P. J.; Shu, L.; Chen, S.; Burdi, D.; Huynh. B. H.; Que, L.; Stubbe, J. "EXAFS Characterization Of the Intermediate X Generated During the Assembly Of E. Coli Ribonucleotide Reductase R2 Differic Tyrosyl Radical Cofactor," *J. Am. Chem. Soc.* **1998**, 120, 849-860.

12. Pulver, S. C.; Tong, T. H.; Bollinger, J. M.; Stubbe, J. Solomon, E. I. "Circular Dichroism and Magnetic Circular Dichroism Studies Of the Fully Reduced Binuclear Non-Heme Iron Active Site in the E. Coli. R2 Subunit of Ribonucleotide Reductase," *J. Am. Chem. Soc.* **1995,** 117, 12664-12678.

13. Lee, D.; De-Bois, J.; Lippard, S. J. "Formation Of Fe(III)Fe(IV) Species From the Reaction Between a Diiron(II) Complex and Dioxygen: Relevance To Ribonucleotide Reductase Intermediate X," *J. Am. Chem. Soc.* **1999,** 121, 9893-9894.