Poster Presentation

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The first ribonucleotide reductase without an activating radical cysteine

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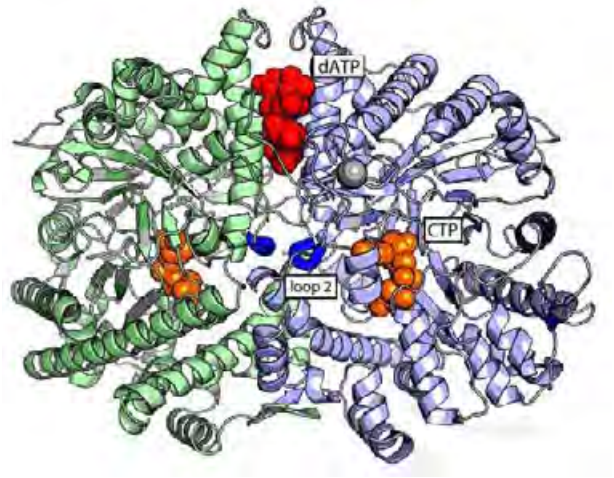
Ribonucleotide reductases (RNRs) catalyze the reduction of ribonucleotides to deoxyribonucleotides, the building blocks for DNA synthesis, and are found in all but a few organisms. RNRs use radical chemistry to catalyze the reduction reaction. Despite RNR having evolved several different mechanisms for generation of different kinds of essential radicals across a large evolutionary time frame, for over 30 years the paradigm has been that this initial radical is always channeled to a strictly conserved cysteine residue directly adjacent to the substrate for initiation of substrate reduction. Such a cysteine residue has been present in the structure of each of the many RNRs determined to date. We present the crystal structure of an anaerobic RNR from the extreme thermophile Thermotoga maritima (tmNrdD), both alone and in complex with allosteric effector dATP and substrate CTP. Remarkably, tmNrdD lacks a cysteine for radical transfer to the substrate, and is the first structurally or biochemically characterized RNR to do so. However in many other respects tmNrdD appears to be a normal anaerobic RNR, including gene structure, expression levels, metal cofactor and binding of allosteric effectors and substrates in the expected conformations. Furthermore, it is possible to generate a glycyl radical as expected. We present evidence that the structure of tmNrdD is representative for the new RNR subclass IIIh, present in all Thermotoga species plus a wider group of bacteria from the distantly related phyla Firmicutes, Bacteroidetes and Proteobacteria, all lacking the canonical cysteine residue. The wide distribution provides further evidence that the subclass IIIh is a functional RNR. Taken together, the results imply that an alternative initiation route for the RNR reduction reaction must exist that do not require channeling through a cysteine side chain.



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