Basis of dATP inhibition of RNRs

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Ribonucleotide reductases (RNRs) are essential enzymes producing de novo deoxynucleotide (dNTP) building blocks for DNA replication and repair and regulating dNTP pools important for fidelity of these processes. A new study reveals that the class 1a Escherichia coli RNR is regulated by dATP via stabilization of an inactive α4β4 quaternary structure, slowing formation of the active α2β2 structure. The results support the importance of the regulatory α4β4 complex providing insight in design of experiments to understand RNR regulation in vivo.

Ribonucleotide reductases (RNRs) are enzymes essential in de novo conversion of nucleotides (UDP, CDP, ADP, and GDP) to deoxynucleotides (dNDPs; dUDP, dCDP, dADP, and dGDP) in all organisms. With rapid subsequent phosphorylation of the dNDPs and conversion of dUDP or dCDP into TTP, RNR provides the monomeric building blocks required for DNA replication and DNA repair. RNRs also play an essential role in controlling the relative ratio and amounts of dNTP pools required for fidelity of these processes (1). The RNR of E. coli is a prototype of the class 1a RNRs (which includes human RNR) and requires two subunits, α and β, each relatively stable homodimers, for activity (Fig. 1). α contains three nucleotide binding sites: the catalytic site (C-site), the specificity site at the α2 interface (S-site), and the activity site (A-site). The A-site is an ATP-cone domain located in the N terminus of α (Fig. 1, green), binds dATP or ATP, and controls RNR activity: ATP activates and dATP inhibits. This inhibition is the subject of a report in this issue of the JBC, Chen et al. (2) highlighted here. β contains a diferri-tyrosyl radical cofactor essential for initiation of the complex radical–dependent reduction chemistry in α. This tyrosyl radical oxidizes a cysteine to a thyl radical in the C-site of α2 > 35 Å away (3)! This process involves long-range radical transfer and is reversible. The active form of E. coli class 1a RNR is described in textbooks as a symmetrical complex of α2β2 and is based on a docking model of high resolution structures of α2 and β2. Biochemical studies have established that the Kd for complex formation between α2 and β2 is weak and nucleotide-dependent. Only when α2, loaded with a matched substrate/effector pair, binds to β2 do conformational changes occur that initiate the reduction chemistry (4). Tight α2β2 interactions occur subsequent to radical initiation and become weak again after each turnover (5).

Structural characterization of the active E. coli RNR α2β2 complex at atomic resolution has remained elusive, but recently, X-ray crystallography and negative stain electron microscopy (EM) have revealed the dATP-inhibited state exhibits an unexpected α4β4 quaternary structure (Fig. 1) (6). Inhibition is thought to arise from a near doubling of the separation between the tyrosyl radical cofactor in β2 and the cysteine in the C-site in α4β4 ( ~ 60 Å) relative to that of α2β2, preventing formation of the essential active site thyl radical required for reduction. Despite the correlation between dATP and inactivation, many questions remain, including the ordering of events that lead to dATP inhibition and the physiological relevance of such activity regulation.

In this issue of the JBC, Chen et al. (2) examine the link between the α4β4 structure, dATP loading, and enzymatic inhibition of E. coli la RNR. Guided by prior structural studies they made mutants of α residues within the α/β interface in the A-site of α4β4 (green in Fig. 1). The mutants were designed to perturb this interaction, while minimizing the effect of dATP/ATP binding to the ATP-cone. Analytical ultracentrifugation (AUC) and negative stain EM methods were used to assess changes in quaternary structure in the presence of dATP (175 μM) and correlated with catalytic activity measurements relative to wt levels. They concluded from their studies that dATP binds to the α4β4 structure and results in RNR inhibition. However, given the distinct concentration dependence of the EM (nm) and AUC (μm) measurements, insight into the Kd for dATP binding for each mutant and for the Kd for α/β interaction in the mutants relative to wt (α/β) would have provided an even stronger correlation between the data sets. The Kd values will also be critical in evaluating how the in vitro model may translate in vivo, where nucleotide pools are complex and dynamic. In the cell, an inhibited α4β4 structure would need to realign with α2β2 in response to changes in the ratio of dATP/ATP pools and deoxynucleotide concentrations.

The conclusions drawn by Chen et al. (2) compliment prior genetic studies of Ahluwalia et al. (7). Using a genetic approach in E. coli, they randomly mutagenized the α and β subunits of
The E. coli 1a RNR and selected for mutator phenotypes with altered dNTP pool ratios and amounts. Interestingly, some of the same residues Chen et al. (2) targeted in α, and additional residues in β not targeted, were identified in this prior study. These residues also mapped to the α/β interface. Both their work and that of Chen et al. (2) provide important support for the biological relevance of α4β4 and its role in controlling dNTP levels in E. coli.

Recently structures of the dATP inhibited human class Ia RNR, a structural homolog to that of E. coli, have been reported (8, 9). Interestingly, in these structures an α6 ring hexamer was observed with no β subunit. This trimer of dimers (Fig. 1) is formed by dATP cone-dATP cone interactions and appears to preclude access of dATP, a requisite for catalysis. Thus, both the dATP inhibited human and E. coli RNRs appear to reduce the rate of formation of the αβ2 complex required for effective radical transfer, but by two distinct mechanisms.

Further support for the importance of dATP control of quaternary structure as the basis of dATP inhibition in human cells is provided by recent studies with clofarabine (CIF). CIF is an adenosine analog that in its triphosphate state inhibits the ternary structure as the basis of dATP inhibition in human cells (2). Interestingly, in these structures an α6 ring hexamer was observed with no β subunit. This trimer of dimers (Fig. 1) is formed by dATP cone-dATP cone interactions and appears to preclude access of dATP, a requisite for catalysis. Thus, both the dATP inhibited human and E. coli RNRs appear to reduce the rate of formation of the αβ2 complex required for effective radical transfer, but by two distinct mechanisms.

The report of Chen et al. (2) and prior biological studies identifying α/β interface residues of α4β4 in E. coli RNR, provide an exciting starting point for understanding class Ia RNR regulation in vivo. Our ability to incorporate fluorescent or cross-linking probes site-specifically into the RNR subunits in vivo will allow monitoring of quaternary structural changes and the dynamics of their interconversions. With new “omics” technologies to both look at nucleotide metabolism and to modulate it via changing genetic backgrounds, we anticipate many new insights to come in this system so central to nucleic acid metabolism.

Figure 1. X-ray structures of class Ia ribonucleotide reductases in their proposed active and dATP-inhibited states. α is shown as blue and cyan (ATP-cone A-site in green), β is shown as red and orange, and the substrate is shown as magenta, with the effector in yellow.

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