7.12: Deoxyribonucleotide de novo Biosynthesis

Synthesis of deoxyribonucleotides de novo requires an interesting enzyme called ribonucleotide reductase (RNR). RNR catalyzes the formation of deoxyribonucleotides from ribonucleotides. The most common form of RNR is the Type I enzyme, whose substrates are ribonucleoside diphosphates (ADP, GDP, CDP, or UDP) and the products are deoxyribonucleoside diphosphates (dADP, dGDP, dCDP, or dUDP). Thymidine nucleotides are synthesized from dUDP. RNR has two pairs of two identical subunits - R1 (large subunit) and R2 (small subunit). R1 has two allosteric binding sites and an active site. R2 forms a tyrosine radical necessary for the reaction mechanism of the enzyme.

Because a single enzyme, RNR, is responsible for the synthesis of all four deoxyribonucleotides, it is necessary to have mechanisms to ensure that the enzyme produces the correct amounts of each dNDP. This means that the enzyme must be responsive to the levels of the each deoxynucleotide, selectively making more of those that are in short supply, and preventing synthesis of those that are abundant. These demands are met by having two separate control mechanisms,

Figure 7.12.1: dNTP de novo Synthesis
one that determines which substrate will be acted on, and another that controls the enzyme’s catalytic activity.

Figure 7.12.2: Thymidylate (dTMP) Synthesis

Ribonucleotide reductase is allosterically regulated via two binding sites - a specificity binding site (binds dNTPs and controls which substrates the enzyme binds and thus, which deoxyribonucleotides are made) and an activity binding site (controls whether or not enzyme is active - ATP activates, dATP inactivates).

When a deoxypyrimidine triphosphate, dTTP is abundant, it binds to the specificity site and inhibits binding and reduction of pyrimidine diphosphates (CDP and UDP) but stimulates binding and reduction of GDP by the enzyme. Conversely, binding of the deoxypurine triphosphate, ATP stimulates reduction of pyrimidine diphosphates, CDP and UDP.

Students sometimes confuse the active site of RNR with the activity site. The active site is where the reaction is catalyzed, and could also be called the catalytic site, whereas the activity site is the allosteric binding site for ATP or dATP that controls whether the enzyme is active.

Synthesis of dTTP by the de novo pathway takes a convoluted pathway from dUDP to dUTP to dUMP to dTMP, then dTDP, and finally dTTP. Conversion of dUMP to dTMP, requires a tetrahydrofolate derivative and the enzyme thymidylate synthase. In the process, dihydrofolate is produced and must be converted back to tetrahyrdolate in order to keep nucleotide synthesis occurring. The enzyme involved in the conversion of dihydrofolate to tetrahydrofolate, dihydrofolate reductase (DHFR), is a target of anticancer drugs like methotrexate or aminopterin, which inhibit the enzyme.

Contributors

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)