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Class I Ribonucleotide Reductases: overall activity regulation, oligomerization, and drug targeting

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i Lilla Hörsalen, KB.E3.01, KBC huset, fredagen den 12 may, kl. 09:00. Avhandlingen kommer att försvaras på engelska.

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Abstract

Ribonucleotide reductase (RNR) is a key enzyme in the *de novo* biosynthesis and homeostatic maintenance of all four DNA building blocks by being able to make deoxyribonucleotides from the corresponding ribonucleotides. It is important for the cell to control the production of a balanced supply of the dNTPs to minimize misincorporations in DNA. Because RNR is the rate-limiting enzyme in DNA synthesis, it is an important target for antimicrobial and antiproliferative molecules. The enzyme RNR has one of the most sophisticated allosteric regulations known in Nature with four allosteric effectors (ATP, dATP, dGTP, and dTTP) and two allosteric sites. One of the sites (s-site) controls the substrate specificity of the enzyme, whereas the other one (a-site) regulates the overall activity. The a-site binds either dATP, which inhibits the enzyme or ATP that activates the enzyme. In eukaryotes, ATP activation is directly through the a-site and in *E. coli* it is a cross-talk effect between the a and s-sites. It is important to study and get more knowledge about the overall activity regulation of RNR, both because it has an important physiological function, but also because it may provide important clues to the design of antibacterial and antiproliferative drugs, which can target RNR.

Previous studies of class I RNRs, the class found in nearly all eukaryotes and many prokaryotes have revealed that the overall activity regulation is dependent on the formation of oligomeric complexes. The class I RNR consists of two subunits, a large α subunit, and a small β subunit. The oligomeric complexes vary between different species with the mammalian and yeast enzymes cycle between structurally different active and inactive $\alpha_6\beta_2$ complexes, and the *E. coli* enzyme cycles between active $\alpha_2\beta_2$ and inactive $\alpha_4\beta_4$ complexes. Because RNR equilibrates between many different oligomeric forms that are not resolved by most conventional methods, we have used a technique termed gas-phase electrophoretic macromolecule analysis (GEMMA). In the present studies, our focus is on characterizing both prokaryotic and mammalian class I RNRs. In one of our projects, we have studied the class I RNR from *Pseudomonas aeruginosa* and found that it represents a novel mechanism of overall activity allosteric regulation, which is different from the two known overall activity allosteric regulation found in *E. coli* and eukaryotic RNRs, respectively. The structural differences between the bacterial and the eukaryote class I RNRs are interesting from a drug developmental viewpoint because they open up the possibility of finding inhibitors that selectively target the pathogens. The biochemical data that we have published in the above project was later supported by crystal structure and solution X-ray scattering data that we published together with Derek T. Logan`s research group.

We have also studied the effect of a novel antiproliferative molecule, NSC73735, on the oligomerization of the human RNR large subunit. This collaborative research results showed that the molecule NSC73735 is the first reported non-nucleoside molecule which alters the oligomerization to inhibit human RNR and the molecule disrupts the cell cycle distribution in human leukemia cells.

Keywords

RNR, allosteric regulation, overall activity regulation, gemma, enzyme activity measurements, nucleotide-filter binding assay, hplc, *P. aeruginosa*

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