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Mechanistic studies of inhibitors of DNA replication restart pathways in *Neisseria gonorrhoeae*

Chaitanya DRK Aduri and Matthew E. Lopper, Ph.D.
Department of Chemistry, University of Dayton, Dayton, OH

Abstract

Complete and faithful replication of a cell’s genetic information is an essential process. Many enzymes are involved in the successful duplication of cells’ genetic information and the integrity of these enzymes can be compromised when they encounter DNA damage. Bacterial cells use a pathway called “DNA replication restart” to repair DNA replication following a disruptive encounter of the DNA replication enzymes with DNA damage. This pathway is catalyzed by primosome proteins, including PriA, PriB, PriC, DnaT, DnaB, DnaC, and DnaG. The importance of DNA replication restart for bacterial cell survival is demonstrated by the inability of strains that carry mutations in key primosome genes to grow and resist DNA damaging agents. Furthermore, this pathway is specific for bacterial cells: human cells don’t use the same replication restart pathway and they don’t encode genes for the primosome proteins that function in bacteria. Since DNA replication restart pathways are essential for bacterial cell growth and survival and are notably absent in human cells, we seek to answer the following question: can bacterial DNA replication restart pathways be targeted with novel antibacterial compounds?

In order to answer this question, we have developed an enzyme-based assay for high-throughput inhibitor screening to identify compounds that block the function of the primosome proteins PriA and PriB. Several interesting lead compounds have already been identified from the preliminary screening. In this study, the lead compounds have been validated as legitimate inhibitors and characterized with respect to their potency and mechanism of action.

DNA replication and DNA replication restart

The restart pathways follow two trends with respect to the origin, origin dependent and origin independent, differing mainly in the mechanistic features. In E. coli, one of the several major pathways to activate stalled replication forks is DnaC-catalyzed origin-dependent initiation of DNA replication. This is very carefully regulated sequence-specific event which is catalyzed by the initiator protein DnaC. DnaC recognizes and binds to a replication origin site and recruits the replicative helicase and the helicase loader protein, DnaB and DnaC respectively.

Origin-independent initiation of DNA replication (known as DNA replication restart) requires a distinct cellular machinery to restart the replication machinery (replication) at a repaired DNA replication fork. This replication process is initiated by an assembly of the primosome proteins, which collectively include PriA, PriB, PriC, DnaT, DnaB, DnaC, DnaG, and Rep proteins.

Effect of 0039

This is a weak inhibitor and is not dependent on PriB.

Effect of 0046

The samples ranging from 300μM – 800μM concentration has shown precipitation.

Effect of 0046

This inhibitor turned out to be a weak inhibitor, and is also not dependent on the PriB.

Identification of inhibitors that selectively inhibit DNA replication restart primosome proteins

We developed an enzyme-based assay to use in high-throughput screening to identify inhibitors of PriA/PriB function.

Synthetic DNA oligonucleotides were used to construct a forked DNA substrate in which the nascent lagging strand arm is fluorescently labeled.

Fluorescence polarization spectroscopy was used to report PriA-PriB-catalyzed unwinding of the forked DNA substrate by monitoring changes in fluorescence anisotropy (FA).

The Life chemicals 2 library was screened to identify inhibitors of PriA-PriB-catalyzed DNA unwinding.

Effect of 0041

This inhibitor had a solubility issue, thus the maximum concentration used in the assay was 50μM.

The inhibitor can be regarded as a weak inhibitor, thus can be taken into account as the one which might be affecting the DNA by itself irrespective of the PriA.

Effect of 0207

This inhibitor looks genuine and the IC50 value was calculated to be 1.84μM.

This indeed is not dependent on PriB.

Precipitation was found in the samples ranging from 100μM and 200μM concentrations.

0207 also shows its effect on E. coli PriA and the IC50 value was calculated to be 1.72μM.

Conclusions

0207 was proved to be a genuine inhibitor.

0207 is not a species specific inhibitor, it also shows its effects on E. coli PriA.

0207 shows mixed mode of inhibition.

References


0207 Affects PriA-catalyzed ATP hydrolysis

- A coupled spectrophotometric assay was used to measure steady-state rates of ATP hydrolysis catalyzed by PriA in the presence and absence of 0207