Understanding the Efficacy of Inhibitor TRDL-551 on Replication Protein A (RPA) Binding Affinity

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Replication protein A (RPA), a single stranded DNA (ssDNA) binding protein, prevents ssDNA from being a target of cellular nucleases, recombining with other complementary sequences, or folding-back on itself to form hairpin structures. Each of these scenarios can cause high rates of genome stability. Due to the protective function of RPA it now used as a therapeutic target for cancer cells, wherein DNA replication and repair often occur at high frequencies. For the current study, we utilized a chemical inhibitor of RPA, TDRL-551, which has been previously characterized as a potent inhibitor of RPA binding to substrate. In this study, we chose to further analyze the TDRL-551 inhibitor by testing different binding conditions either by the unmodified or acetylated form of RPA. Results from our laboratory have shown that acetylated RPA binds with higher affinity to ssDNA compared to the unmodified form. We tested RPA (unmodified and acetylated) binding under two different conditions; (i) RPA was pre-bound to substrate and then incubated with inhibitor or (ii) RPA was pre-bound to inhibitor and then exposed to substrate. Our results showed that in the first case, there was no effect of exposure to inhibitor on both unmodified and acetylated RPA binding. However, when RPA was first incubated with the inhibitor, both the unmodified and acetylated RPA showed reduced binding affinity to the ssDNA substrate. In conclusion, our studies show that the length of the DNA substrate, the post-translational status of RPA and the time of exposure of the inhibitor to the RPA all play a significant role in determining the potency of this inhibitor. Further studies are being done to ascertain the efficacy of this inhibitor before using it in translational research.

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