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CHARACTERIZATION OF NUCLEOTIDE BINDING DURING THE ELONGATION PHASE OF HEPATITIS C RNA SYNTHESIS

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The hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase is essential for viral replication and is a major target for current drug development efforts. RNA synthesis by the polymerase occurs via a *de novo* mechanism or by the use of short primers. Synthesis is distributive at the initiation stage and becomes processive as the enzyme undergoes conformational changes and enters the elongation phase. Replication can be inhibited by nucleoside analogues that compete with natural nucleotides for incorporation. Inhibitors of this class that have advanced to clinical trials include the prodrugs of 2′-C-methylcytidine and 4′-azidocytidine. Treatment of HCV replicon-containing cells with these compounds selects for the resistance-conferring mutations S282T and S96T, respectively. While nucleotide incorporation has been studied at the initiation phase, detailed studies at the elongation stage have not been undertaken. Here, we studied single-nucleotide incorporation during elongation by wild-type (WT) NS5B an enzyme harbouring the S282T mutation. Previous reports on the binding affinity for the incoming nucleotide during initiation gave a dissociation constant (K_d) in the low micromolar range. In our studies focusing on the elongation phase, the K_d value for CTP was in the low nanomolar range, suggesting that binding affinity for nucleotides by the enzyme-nucleic acid complex is much higher at this step. Binding of the nucleotide analogue 2′-C-methylcytidine by the enzyme was also very efficient, with a K_d value only 2-fold higher than for WT. K_d values for CTP incorporation by the S282T mutant were similar to those obtained for the WT enzyme, and, as expected, binding of 2′-C-methylcytidine by the S282T mutant was severely impaired. Due to the error-prone nature of the polymerase, we compared binding of mismatched nucleotides during elongation with binding of the correct nucleotide and the nucleotide analogue. The K_d value for binding of UTP opposite template G was in the low micromolar range, which is also low when compared with the fidelity of other polymerases. However, this value is still high when compared with the binding properties of the inhibitor. Together, these data suggest that the inhibition of RNA synthesis by 2′-methylated nucleotide analogues can be more efficient then mismatch formation.