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The Macroscopic Rate of Nucleic Acid Translocation by Hepatitis C Virus Helicase NS3h Is Dependent on Both Sugar and Base Moieties

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The nonstructural protein 3 helicase (NS3h) of hepatitis C virus is a 3′-to-5′ superfamily 2 RNA and DNA helicase that is essential for the replication of hepatitis C virus. We have examined the kinetic mechanism of the translocation of NS3h along single-stranded nucleic acid with bases uridylate (rU), deoxyuridylate (dU), and deoxythymidylate (dT), and have found that the macroscopic rate of translocation is dependent on both the base moiety and the sugar moiety of the nucleic acid, with approximate macroscopic translocation rates of 3 nt s^{-1} (oligo(dT)), 35 nt s^{-1} (oligo(dU)), and 42 nt s^{-1} (oligo(rU)), respectively. We found a strong correlation between the macroscopic translocation rates and the binding affinity of the translocating NS3h protein for the respective substrates such that weaker affinity corresponded to faster translocation. The values of $K_{0.5}$ for NS3h translocation at a saturating ATP concentration are as follows: 3.3±0.4 μM nucleotide (poly(dT)), 27±2 μM nucleotide (poly(dU)), and 36±2 μM nucleotide (poly(rU)). Furthermore, results of the isothermal titration of NS3h with these oligonucleotides suggest that differences in $T\Delta S^0$ are the principal source of differences in the affinity of NS3h binding to these substrates. Interestingly, despite the differences in macroscopic translocation rates and binding affinities, the ATP coupling stoichiometries for NS3h translocation were identical for all three substrates (~0.5 ATP molecule consumed per nucleotide translocated). This similar periodicity of ATP consumption implies a similar mechanism for NS3h translocation along RNA and DNA substrates.

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Keywords: NS3 helicase; translocation; nucleic acid; ATPase; mechanism

Introduction

Helicases are motor proteins that utilize the energy generated through ATP binding and hydrolysis for translocation along single-stranded nucleic acids and unwinding of double-stranded nucleic acids.[1–5] Members of this ubiquitous class of proteins play a vital role in DNA and RNA metabolism, participating in many critical processes including replication, recombination, and repair.[6,7] Hepatitis C virus (HCV) is a single-stranded RNA virus that requires several virally encoded proteins for successful replication. One of the proteins that is essential for viral replication is nonstructural protein 3 (NS3).[8] NS3 is a multifunctional protein that possesses N-terminal protease activity and C-terminal NTPase and helicase activities.[9,10] Both protease and helicase activities are required for viral replication.[11] The helicase domain of NS3 is a member of helicase superfamily 2[12] and is capable of unwinding both RNA and DNA[13] duplexes with a 3′-to-5′ directional bias[9,12,13] and translocating along single-stranded DNA with an identical 3′-to-5′ directional bias.[14]

Based on the initial structural study performed on the helicase domain of NS3 [nonstructural protein 3

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Abbreviations used: NS3h, nonstructural protein 3 helicase; HCV, hepatitis C virus; NS3, nonstructural protein 3; Mops, morpholinepropanesulfonic acid; NLLS, nonlinear least squares; ITC, isothermal titration calorimetry; CV, column volumes.

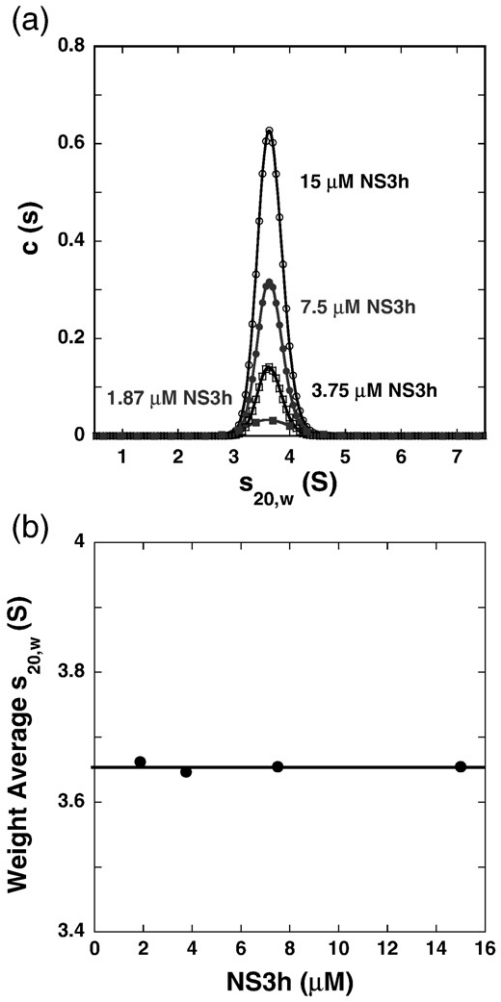

Fig. 1. NS3h is a monomer in solution. (a) The $c(s)$ distributions resulting from the analysis of sedimentation velocity experiments conducted at 25 °C and 42,000 rpm in translocation buffer with four different total NS3h concentrations: 15 μM (black open circles), 7.5 μM (gray filled circles), 3.75 μM (black open squares), and 1.87 μM (gray filled squares). NS3h absorbance measured at 280 nm. (b) The weight-average estimate of $s_{20,w}$ calculated from further analysis of the data in (a) is independent of the total concentration of NS3h in the reaction. Taken together, these data are consistent with NS3h existing as a homogenous stable monomer in solution.

helicase (NS3h)], it was suggested that previously observed differences in the binding affinity of NS3h for a series of short oligonucleotides and the corresponding differences in the stimulation of the ATPase activity of NS3h in the presence of these same oligonucleotides were, to a large extent, dominated by differences in entropic contributions to the free energies of NS3h binding to these substrates.[15] Furthermore, based on the observation that the oligonucleotide bound to NS3h in their crystal structure was slightly bent, these same authors suggested that these differences in the entropic contribution to the free energies of binding are associated with deformation of the substrates themselves.[15] More recent crystal structures of NS3h bound to oligo(deoxyuridylate) (dU) showed that the contacts between NS3h and the nucleic acid were largely limited to the phosphate backbone.[16] These studies revealed three distinct crystals of NS3h together with single-stranded DNA and different ATP analogs, leading to the proposal of a physical model for DNA translocation by NS3h, where changes in the intradomain structure of the enzyme were directly coupled to the structural transitions of the associated DNA.[16] Based on this model, these authors further speculated that the preference of the ribose sugar to adopt a C3′-endo sugar pucker rather than a C2′-endo sugar pucker would favor NS3h binding to DNA over NS3h binding to RNA. Subsequently, based on their results, one would expect that these differences in NS3h binding would translate into variability in translocation behavior along DNA and RNA.

Recent biochemical studies on NS3-catalyzed nucleic acid unwinding have been used to explain nucleic acid translocation by NS3,[17–21] although a clear mechanism of action for either of these processes has not been identified. Two primary models have been used to describe the mechanism of unwinding and translocation by NS3. The first model is a brownian motor mechanism for unwinding and translocation, which is based on NS3h substrate binding studies in the presence and in the absence of ATP.[18,19] The second model is the more commonly accepted inchworm model, initially suggested in lieu of structural evidence.[15] Recent biochemical studies with full-length NS3 using single-molecule fluorescence resonance energy transfer and optical tweezers have also supported this model.[17,20,21] Furthermore, a similar model has been suggested for the SF1 helicase PcrA.[22–24] Experiments performed for NS3 assuming the inchworm model have identified large and small step sizes of 11–18 bp[17,21] and 3 bp,[17,20] respectively. The small step size was further shown to consist of three 1-bp "hidden steps." Therefore, each 1-bp step was shown to correspond to the hydrolysis of one ATP.[20] A large physical step size for single-stranded nucleic acid translocation is also suggested by the fact that NS3 has been shown to tolerate large disruptions in its nucleic acid tracking strand during double-stranded nucleic acid unwinding.[25]

In order to better understand the mechanism of action of this helicase, we performed a series of pre-steady-state stopped-flow experiments to determine the macroscopic translocation rates along 5′ fluorescently labeled single-stranded oligonucleotides. In this study, we utilized various lengths of oligo (deoxythymidylate) (dT), oligo(dU), and oligo(uridylate) (rU) to measure the macroscopic translocation rates and coordinated these rates with ATP consumption based on ATPase stimulation experiments using poly(dT), poly(dU), and poly(rU) substrates. Lastly, we independently determined the K_d for NS3h binding to poly(dT) and poly(dU) to further complement translocation experiments. The work presented here is the first reported rigorous