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Bacteriophage T7 Replicative DNA Helicase: A Molecular Motor Translocating Along Single-Stranded DNA

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Introduction

DNA helicases are molecular motors that use the energy from NTP hydrolysis to drive the process of duplex DNA strand separation. Most helicases require and interact with ssDNA regions flanking one end of the dsDNA during DNA unwinding. Several studies suggest that helicases can translocate along ssDNA in a unidirectional manner[1-4]. Yet, it is not known whether translocation along ssDNA is an efficient process in terms of processivity and rate and thus it is not clear whether it plays a major role in DNA unwinding. The possibility remains that helicases require interactions with the ss/ds DNA junction for efficient unidirectional movement along DNA.

The T7 helicases belong to the class of helicases that assemble into hexameric rings, a structure that is required for the helicase reaction[5,6] (Fig. 1A). T7 helicase hydrolyzes various NTPs, but hydrolysis of dTTP is most efficiently coupled to the process of DNA strand separation[7,8]. The dTTPase activity of T7 helicase is greatly stimulated by the presence of ssDNA[8]. Studies of this and other hexameric helicases support a DNA-exclusion model, in which helicase binds only one strand of the duplex DNA through the central channel of the ring and the complementary strand is excluded during DNA strand separation[9,10] (Fig. 1B).

Results and Discussion

Presteady State Kinetics of dTTP Hydrolysis as a Function of Oligo(dT) Length

The presteady state kinetics of dTTP hydrolysis in the presence of ssDNA was measured by following the production of inorganic phosphate (P_i) (one of the products of the dTTPase reaction) in real time using a fluorescent sensor. The fluorescent sensor is a coumarin-labeled phosphate binding protein (PBP-MDCC), whose fluorescence intensity increases ~6 fold upon binding to P_i[11]. A ternary complex of T7 helicase•dTTP•ssDNA was preformed and the dTTPase reaction was initiated by addition of Mg^{2+} in a stopped-flow instrument at 18°C[12]. The resulting P_i-release kinetics are shown in Figure 2. No kinetic lags were observed indicating that Mg^{2+} binding is fast. The P_i-release kinetics was biphasic; that is, the production of P_i or the hydrolysis of dTTP occurs at a fast rate initially, and after some time, the rate decreases to the familiar steady state dTTPase rate. The fast phase therefore represents the presteady state phase of the P_i-release kinetics.

We propose that the presteady state phase represent the P_i-release kinetics of the helicase molecules translocating along ssDNA. The steady state P_i-release kinetics is slower because it is limited by an event that occurs after the helicase reaches the end of linear ssDNA. This may be either the dissociation of the helicase from the end of the ssDNA or the rebinding of the helicase hexamer to ssDNA, which was shown previously to occur at a slow rate [13].

If dTTP hydrolysis in the fast phase is indeed coupled to translocation along ssDNA, then its amplitude,

which represents the number of dTTP molecules hydrolyzed during translocation, should increase with increasing ssDNA length. The P_i-release kinetics was measured using various lengths of oligo(dT) ssDNA (dT_n, n ranging from 40 to 120 bases). As shown in Figure 2, P_i-release occurs with biphasic kinetics at all lengths of dT_n, which were fit to the following equation to obtain the rate and amplitude of the fast phase as well as the rate of the slow phase. $Y = A[1\text{-}exp(-kt)] + mt$, where y is observed fluorescence or molar amount of P_i released at time t, A and k are amplitude and rate of the burst phase, respectively; and m is the rate of the linear steady-state phase.

Kinetic Simulation and Global Fitting of the P_i-release Kinetics to the ssDNA Translocation Mechanism

In order to accurately determine the rate of helicase translocation along ssDNA and the energy coupling efficiency, the P_i-release kinetics were fit to the model shown in Scheme I. This model describes the steps that occur under the experimental conditions from helicase binding to ssDNA to translocation along ssDNA and recycling as well as coupling of these events to dTTPase. The helicase hexamer (Ef) binds ssDNA at random positions on the ssDNA lattice of L bases with a bimolecular rate constant L^*k_{on} to form the species, E0. Previous kinetic studies of ssDNA binding to T7 helicase hexamer have shown that the ssDNA binding process leading to a dTTPase-competent species is a multistep process including a ring-opening step [13]. Upon activation, the helicase hexamer translocates unidirectionally to the end of the ssDNA with an intrinsic translocation rate constant, k_t, to form the species E2. Unidirectional translocation of T7 helicase along ssDNA is supported by the fact that the amplitude and duration time of dTTPase activity in the fast phase increases linearly with ssDNA length.

The helicase may dissociate from the ssDNA before reaching the end with a rate constant, k_d. This parameter (k_d) was directly determined by measuring the dissociation rate constant of fluorescently labeled 30-mer DNA from the T7 hexameric helicase. On the other hand, the dissociation rate of the helicase from the end occurs with a rate constant, k_{end}. We used M13 ssDNA to obtain the dissociation rate constant (k_{end}) of T7 helicase while translocating along ssDNA, in which the dissociation rate was measured by kinetically monitoring the amount of T7 helicase remaining on the closed circular M13 ssDNA.

The free helicase then rebinds the DNA to repeat the next cycle from E0 to E2. The translocating species (E1) hydrolyzes dTTP at the DNA-stimulated rate constant, k_s, and we assign the helicase bound to the end of the DNA (E2) a hydrolysis rate, k_h, and the free helicase or DNA-unbound species (Ef) and the pre-activated species (E0) hydrolyze dTTP at an intrinsic rate constant k_i. Thus, the formation of P_i at any given time is described with the following differential equation; $dP/dt = k_s\,E1 + k_h\,E2 + k_i\,(E0 + Ef)$.

Global fitting was carried out using data sets of P_i-release kinetics measured using ssDNA of increasing lengths shown in Figure 2. Best fit was obtained when the average length of ssDNA that T7 helicase traverses along was assigned a value of half the ssDNA length (L) minus the length occluded by T7 helicase hexamer (A), that is $(L\text{-}A)/2$. The best-fit parameters are summarized in Table 1 with standard deviation for each fitting variable.

Global fitting of the kinetic data provided an average translocation rate of 132 bases per second per hexamer at 18 ^{o}C. While translocating along ssDNA, T7 helicase hydrolyzes dTTP at a rate of 49 dTTP per second per hexamer, which indicates that the energy from hydrolysis of one dTTP drives unidirectional movement of T7 helicase along two to three bases of ssDNA. One of the features that distinguishes this ring helicase is its processivity, which was determined to be 0.99996, indicating that T7 helicase travels on an average about 75kb of ssDNA before dissociating. We propose that the ability of T7 helicase to translocate unidirectionally along ssDNA in an efficient manner plays a crucial role in DNA unwinding.

References

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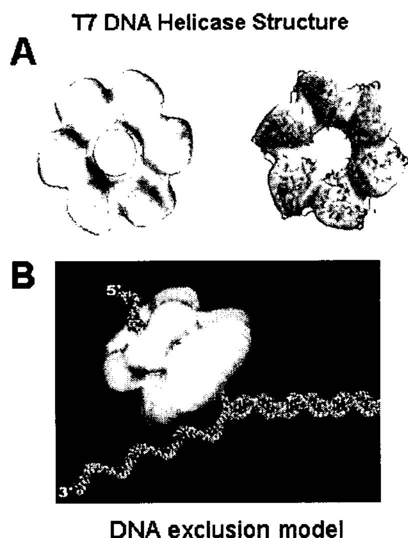


Fig. 1. Structure of T7 DNA Helicase and the DNA exclusion model.

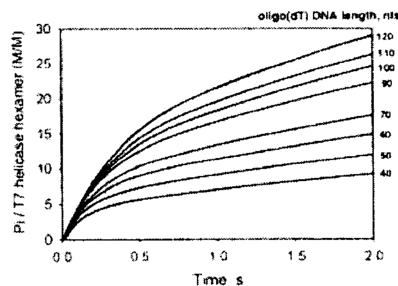
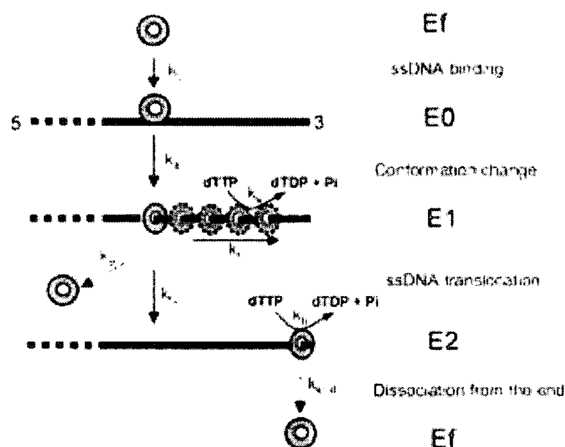


Fig. 2. Presteady state kinetics of P_i-release with ssDNA of various lengths.



Scheme 1. Kinetic pathway of T7 DNA helicase in ssDNA binding and translocation

Table 1. Rate constants of T7 helicase translocation along ssDNA

Step	Reaction	Rate constant
k_{on}	ssDNA binding (Ef → E0)	0.40 ± 0.16 $\mu M^{-1} s^{-1}$
k_a	conformational change (E0 → E1)	0.41 ± 0.007 s^{-1}
k_t	translocation rate (E1 → E2)	132 ± 0.9 s^{-1}
k_d	dissociation during translocation (E1 → Ef)	0.0018 ± 0.0006 s^{-1}
k_{end}	dissociation from ssDNA end (E2 → Ef)	3.7 ± 0.26 s^{-1}
k_s	ssDNAstimulated dTTP hydrolysis	48.8 ± 0.3 dTTP/s/hexamer
k_h	dTTP hydrolysis at ssDNA end	15.3 ± 1.3 dTTP/s/hexamer
k_i	intrinsic dTTP hydrolysis by free T7 helicase	0.7 ± 0.3 dTTP/s/hexamer