SUPPLEMENTARY MATERIAL of "Dda helicase tightly couples translocation on single-stranded DNA to unwinding of duplex DNA: Dda is an optimally active helicase"

Alicia K. Byrd, Dennis L. Matlock, Debjani Bagchi, Suja Aarattuthodiyil, David Harrison, Vincent Croquette, and Kevin D. Raney

The second phase of the association reaction of Dda and DNA is independent of the concentration of DNA, while the rate of the first phase increases as the DNA concentration increases. This indicates that the first phase is association of DNA and Dda (Table S1). The second phase that is insensitive to increasing DNA concentrations likely corresponds to a conformational change that occurs after association of Dda with DNA.

In order to monitor Dda dissociation from DNA, a protein trap is necessary to prevent Dda from rebinding to the DNA after dissociation. The fluorescence of Dda when bound to the trap must be different than when bound to DNA so that the difference in the fluorescence as Dda dissociates from the DNA and binds to the trap can be observed. Heparin was used as a protein trap for the experiments described here. Fig. S1 shows that the fluorescence of Dda at 340 nm is quenched upon binding to DNA. Titration of the DNA-bound enzyme with heparin, leads to a return of the fluorescence to the initial baseline. Since the fluorescence of Dda is distinctly different when bound to DNA *versus* heparin, heparin is an effective protein trap.

Upon mixing of Dda bound ssDNA with heparin, a rapid fluorescence increase is observed. This is independent of the concentrations of Dda, heparin, and DNA but occurs only in the presence of DNA (Table S2), suggesting that it represents dissociation of Dda from DNA and association with heparin.

The rate of Dda dissociation from linear DNA is slower in the absence of ATP than in the presence due to the lack of translocation off the end of the oligonucleotide. In the absence of ATP (Fig. S2) Dda dissociates from a 15mer, 30mer, 45mer, and 60mer at $2.13\pm0.18\ s^{-1}$, $2.40\pm0.90\ s^{-1}$, $1.57\pm0.19\ s^{-1}$, and $1.08\pm0.01\ s^{-1}$, respectively. The small increase in the dissociation rate as the oligonucleotide length decreases suggests that Dda has less affinity for shorter oligonucleotides. This is consistent with the higher equilibrium dissociation constants measured for shorter substrates[1].

The rate of the fluorescence change upon dissociation of Dda from ssDNA is dependent on the length of the DNA substrate (Fig. 2, main text) and concentration of ATP (Table S3). The K_M for ATP for Dda is 92 μM[2]. At ATP concentrations near the K_M, the binding of ATP appears to be rate limiting, while at ATP concentrations well above the K_M, the rate remains constant (Table S3). The results indicate that 600 μM ATP is sufficient to saturate the rate. The rate increases as the concentration of ATP increases (when below or near the K_M); this is to be expected for the dissociation event because in the presence of ATP, Dda is able to translocate, and translocation off the end of the oligonucleotide should increase the observed dissociation rate.

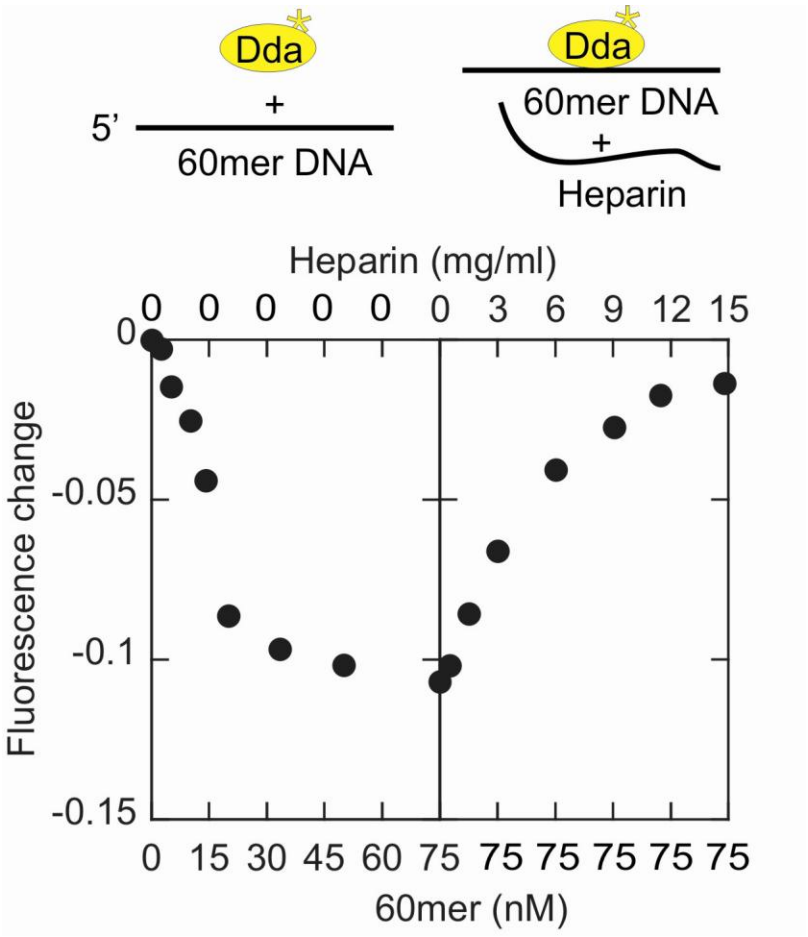

Fig. S1 The fluorescence of Dda is different when bound to DNA and heparin.
200 nM Dda was titrated with 60mer at 25 °C in an SLM Amnico-Bowman fluorescence spectrometer until the fluorescence at 340 nm remained constant, followed by titration with heparin until the fluorescence was again constant. Dda was excited at 280±2 nm and emission was monitored at 340±4 nm. A cuvette with an equal quantity of tryptophan was simultaneously titrated as a reference. The fluorescence of Dda when bound to heparin is more similar to that of free Dda, than that of Dda bound to DNA, indicating that heparin can serve to trap Dda that dissociates from the DNA. Heparin does not affect the Dda dissociation rate (Table S2).

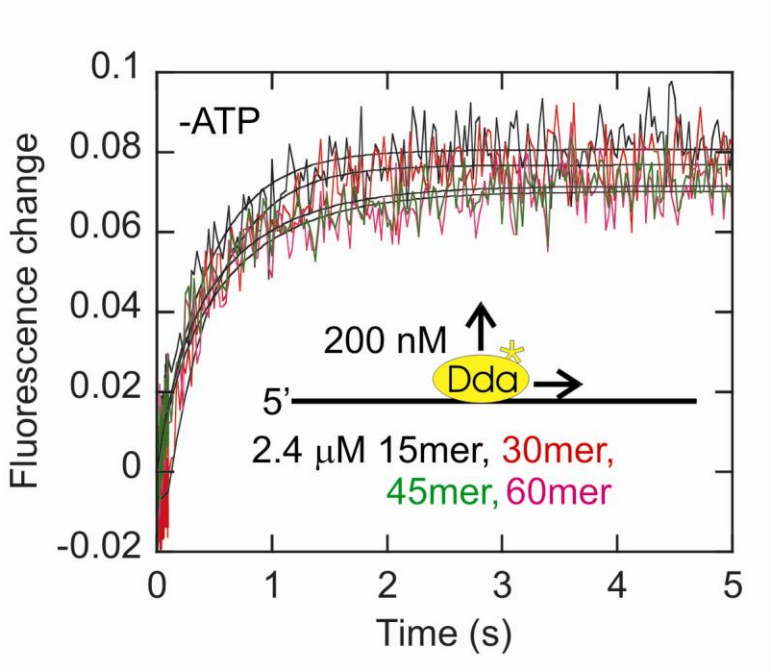

Fig S2 Measurement of the dissociation rate of Dda from ssDNA in the absence of ATP. The change in fluorescence of Dda (200 nM) as it dissociates from 2.4 μM oligonucleotide of increasing length in the absence of ATP is plotted. The rate constants obtained from exponential fits were 2.13±0.18 s^{-1}, 2.40±0.90 s^{-1}, 1.57±0.19 s^{-1}, and 1.08±0.01 s^{-1} for dissociation from 15mer, 30mer, 45mer, and 60mer, respectively.

Table S1 The second phase of the association reaction is independent of DNA concentration, but the rate of the first phase increases as the DNA concentration increases (at 200 nM Dda).

poly(dT) (μM[a])	6	12	15	18
1st phase rate (s^{-1})	163±20	287±32	425±65	518±66
2nd phase rate (s^{-1})	2.65±0.03	2.74±0.24	2.49±0.07	2.41±0.07

[a]in nucleotides

Table S2 The fluorescence change in the dissociation reaction occurs only in the presence of DNA, and the rate is independent of the Dda, heparin, and DNA concentrations.

60mer (μM[a])	-	1.2	2.4	4.8
Dda (nM)	200	100	200	400
heparin (mg/ml)	12	6	12	25
rate (s^{-1})	n.a.[b]	8.1±0.6	8.9±0.50	8.5±0.9

[a]in nucleotides
[b]not applicable

Table S3 The dissociation rate is dependent on the ATP concentration (200 nM Dda, 2.4 μM 60mer in nucleotides).

ATP (μM)	-	50	100	600	900
rate (s^{-1})	1.08±0.01	1.81±0.02	4.88±0.01	8.95±0.50	8.44±0.53

References

1. Byrd, A. K. & Raney, K. D. (2005). Increasing the length of the single-stranded overhang enhances unwinding of duplex DNA by bacteriophage T4 Dda helicase. *Biochemistry* **44**, 12990-12997.
2. Nanduri, B., Eoff, R. L., Tackett, A. J. & Raney, K. D. (2001). Measurement of steady-state kinetic parameters for DNA unwinding by the bacteriophage T4 Dda helicase: use of peptide nucleic acids to trap single-stranded DNA products of helicase reactions. *Nucleic Acids Res.* **29**, 2829-2835.