

Supplemental Material

The Kinetic Mechanism for DNA Unwinding by Multiple Molecules of Dda Helicase Aligned on DNA[†]

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Running title: DNA unwinding by multiple helicases aligned on DNA

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Abbreviations: RQF – Rapid Quench Flow; NLLS – non-linear least squares.

Mechanisms for data fitting using the program Kinetic Global Kinetic Explorer.

Mechanisms were written using the Model Editor of the program according to the following definitions:

- D1: DNA substrate
- D2: DNA substrate after one kinetic step for unwinding
- D3: DNA substrate after two kinetic steps for unwinding
- D4: DNA substrate after three kinetic steps for unwinding
- P: DNA substrate after sufficient number of kinetic steps for ssDNA to form
- E: One molecule of Dda
- EE: Two molecules of Dda
- EEE: Three molecules of Dda
- EEEE: Four molecules of Dda
- A: The trailing molecule of Dda after dissociation of the leading molecule (see Fig. 2 for explanation of the trailing molecule)
- B: The trailing molecule of Dda after one kinetic step for translocation

DNA unwinding of the 14T16bp substrate in 3 kinetic steps with three Dda molecules.

ED1 = ED2 = ED3 = EP
ED1 = E + D1
ED2 = E + D2
ED3 = E + D3
EED1 = EED2 = EED3 = EEP
EED1 = ED1
EED2 = ED2
EED3 = ED3
EED1 = E + AD1
EED2 = E + AD2
EED3 = E + AD3
AD1 = BD1
AD2 = BD2
AD3 = BD3
BD1 = ED1
BD2 = ED2
BD3 = ED3
AD1 = E + D1
AD2 = E + D2
AD3 = E + D3
BD1 = E + D1
BD2 = E + D2
BD3 = E + D3

$\text{Chi}^2 = 0.01377$
 $\text{Chi}^2/\text{DoF} = 0.001059$
 $\text{Sigma} = 0.0325$

DNA unwinding of the 14T20bp substrate in 4 kinetic steps with two Dda molecules.

$$ED1 = ED2 = ED3 = ED4 = EP$$

$$ED1 = E + D1$$

$$ED2 = E + D2$$

$$ED3 = E + D3$$

$$ED4 = E + D4$$

$$EED1 = EED2 = EED3 = EED4 = EEP$$

$$EED1 = ED1$$

$$EED2 = ED2$$

$$EED3 = ED3$$

$$EED4 = ED4$$

$$EED1 = E + AD1$$

$$EED2 = E + AD2$$

$$EED3 = E + AD3$$

$$EED4 = E + AD4$$

$$AD1 = BD1$$

$$AD2 = BD2$$

$$AD3 = BD3$$

$$AD4 = BD4$$

$$BD1 = ED1$$

$$BD2 = ED2$$

$$BD3 = ED3$$

$$BD4 = ED4$$

$$AD1 = E + D1$$

$$AD2 = E + D2$$

$$AD3 = E + D3$$

$$AD4 = E + D4$$

$$BD1 = E + D1$$

$$BD2 = E + D2$$

$$BD3 = E + D3$$

$$BD4 = E + D4$$

$$\text{Chi}^2 = 0.00350236$$

$$\text{Chi}^2/\text{DoF} = 0.000269412$$

$$\text{Sigma} = 0.0164$$

DNA unwinding of the 21T16bp substrate in 3 kinetic steps with three Dda molecules.

$$ED1 = ED2 = ED3 = EP$$

$$ED1 = E + D1$$

$$ED2 = E + D2$$

$$ED3 = E + D3$$

$$EED1 = EED2 = EED3 = EEP$$

$$EED1 = ED1$$

$$EED2 = ED2$$

$$EED3 = ED3$$

$$EEED1 = EEED2 = EED3 = EEEP$$

$$EEED1 = E + EED1$$

$$EEED2 = E + EED2$$

$$EEED3 = E + EED3$$

$$EEED1 = EAD1$$

$$EEED2 = EAD2$$

$$EEED3 = EAD3$$

$$EED1 = E + AD1$$

$$EED2 = E + AD2$$

$$EED3 = E + AD3$$

$$EAD1 = EED1$$

$$EAD2 = EED2$$

$$EAD3 = EED3$$

$$AD1 = BD1$$

$$AD2 = BD2$$

$$AD3 = BD3$$

$$BD1 = ED1$$

$$BD2 = ED2$$

$$BD3 = ED3$$

$$AD1 = E + D1$$

$$AD2 = E + D2$$

$$AD3 = E + D3$$

$$BD1 = E + D1$$

$$BD2 = E + D2$$

$$BD3 = E + D3$$

$$\text{Chi}^2 = 0.0221537$$

$$\text{Ch}^2/\text{DoF} = 0.00170413$$

$$\text{Sigma} = 0.0412811$$

DNA unwinding of the 21T20bp substrate in 3 kinetic steps with three Dda molecules.

$$ED1 = ED2 = ED3 = ED4 = EP$$

$$ED1 = E + D1$$

$$ED2 = E + D2$$

$$ED3 = E + D3$$

$$ED4 = E + D4$$

$$EED1 = EED2 = EED3 = EED4 = EEP$$

$$EED1 = E + ED1$$

$$EED2 = E + ED2$$

$$EED3 = E + ED3$$

$$EED4 = E + ED4$$

$$EEED1 = EEED2 = EEED3 = EEED4 = EEEP$$

$$EEED1 = E + EED1$$

$$EEED2 = E + EED2$$

$$EEED3 = E + EED3$$

$$EEED4 = E + EED4$$

$$EEED1 = EAD1$$

$$EEED2 = EAD2$$

$$EEED3 = EAD3$$

$$EEED4 = EAD4$$

$$EED1 = E + AD1$$

$$EED2 = E + AD2$$

$$EED3 = E + AD3$$

$$EED4 = E + AD4$$

$$EAD1 = EBD1 = EED1$$

$$EAD2 = EBD2 = EED2$$

$$EAD3 = EBD3 = EED3$$

$$EAD4 = EBD4 = EED4$$

$$AD1 = BD1 = ED1$$

$$AD2 = BD2 = ED2$$

$$AD3 = BD3 = ED3$$

$$AD4 = BD4 = ED4$$

$$AD1 = E + D1$$

$$AD2 = E + D2$$

$$AD3 = E + D3$$

$$AD4 = E + D4$$

$$BD1 = E + D1$$

$$BD2 = E + D2$$

$$BD3 = E + D3$$

$$BD4 = E + D4$$

$$\text{Chi}^2 = 0.0121836$$

$$\text{Ch}^2/\text{DoF} = 0.000937204$$

$$\text{Sigma} = 0.0306138$$

DNA unwinding of the 28T16bp substrate in 3 kinetic steps with four Dda molecules.

$$ED1 = ED2 = ED3 = EP$$

$$ED1 = E + D1$$

$$ED2 = E + D2$$

$$ED3 = E + D3$$

$$EED1 = EED2 = EED3 = EEP$$

$$EED1 = ED1$$

$$EED2 = ED2$$

$$EED3 = ED3$$

$$EEED1 = EEED2 = EEED3 = EEEP$$

$$EEED1 = E + EED1$$

$$EEED2 = E + EED2$$

$$EEED3 = E + EED3$$

$$EEEEED1 = EEEEEED2 = EEEEEED3 = EEEEEEP$$

$$EEEEED1 = E + EEED1$$

$$EEEEED2 = E + EEED2$$

$$EEEEED3 = E + EEED3$$

$$EEED1 = EAD1$$

$$EEED2 = EAD2$$

$$EEED3 = EAD3$$

$$EEEEED1 = EEAD1$$

$$EEEEED2 = EEAD2$$

$$EEEEED3 = EEAD3$$

$$EEAD1 = EEED1 = EEED1$$

$$EEAD2 = EEED2 = EEED2$$

$$EEAD3 = EEED3 = EEED3$$

$$EED1 = E + AD1$$

$$EED2 = E + AD2$$

$$EED3 = E + AD3$$

$$EAD1 = EBD1 = EED1$$

$$EAD2 = EBD2 = EED2$$

$$EAD3 = EBD3 = EED3$$

$$AD1 = BD1$$

$$AD2 = BD2$$

$$AD3 = BD3$$

$$BD1 = ED1$$

$$BD2 = ED2$$

$$BD3 = ED3$$

$$AD1 = E + D1$$

$$AD2 = E + D2$$

$$AD3 = E + D3$$

$$BD1 = E + D1$$

$$BD2 = E + D2$$

$$BD3 = E + D3$$

$$\text{Chi}^2 = 0.0263758$$

$$\text{Ch}^2/\text{DoF} = 0.00219798$$

$$\text{Sigma} = 0.0468826$$

Mechanism for DNA unwinding of the 28T16bp substrate in 3 kinetic steps.

ED1 = ED2 = ED3 = ED4 = EP
ED1 = E + D1
ED2 = E + D2
ED3 = E + D3
ED4 = E + D4
EED1 = EED2 = EED3 = EED4 = EEP
EED1 = ED1
EED2 = ED2
EED3 = ED3
EED4 = ED4
EEED1 = EEED2 = EEED3 = EEED4 = EEEP
EEED1 = E + EED1
EEED2 = E + EED2
EEED3 = E + EED3
EEED4 = E + EED4
EEEEED1 = EEEEEED2 = EEEEEED3 = EEEEEED4 = EEEEEEP
EEEEED1 = E + EEEEEED1
EEEEED2 = E + EEEEEED2
EEEEED3 = E + EEEEEED3
EEEEED4 = E + EEEEEED4
EEED1 = EAD1
EEED2 = EAD2
EEED3 = EAD3
EEED4 = EAD4
EEEEED1 = EEAD1
EEEEED2 = EEAD2
EEEEED3 = EEAD3
EEEEED4 = EEAD4
EEAD1 = EEBD1 = EEED1
EEAD2 = EEBD2 = EEED2
EEAD3 = EEBD3 = EEED3
EED1 = E + AD1
EED2 = E + AD2
EED3 = E + AD3
EAD1 = EBD1 = EED1
EAD2 = EBD2 = EED2
EAD3 = EBD3 = EED3
AD1 = BD1
AD2 = BD2
AD3 = BD3
BD1 = ED1
BD2 = ED2
BD3 = ED3
AD1 = E + D1
AD2 = E + D2
AD3 = E + D3
BD1 = E + D1
BD2 = E + D2
BD3 = E + D3

$$\text{Chi}^2 = 0.00912995$$

$$\text{Ch}^2/\text{DoF} = 0.000702303$$

$$\text{Sigma} = 0.026501$$