Kinetic investigation of the polymerase and exonuclease activities of human DNA polymerase epsilon holoenzyme

Walter J. Zahurancik[a] and Zucai Suo[a,b,*]

[a]The Ohio State Biochemistry Program, The Ohio State University, Columbus, OH 43210, USA.

[b]Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL 32306, USA

*To whom correspondence should be addressed: Zucai Suo, Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL 32306, USA; Tel.: (850) 645-2501; E-mail: zucai.suo@med.fsu.edu

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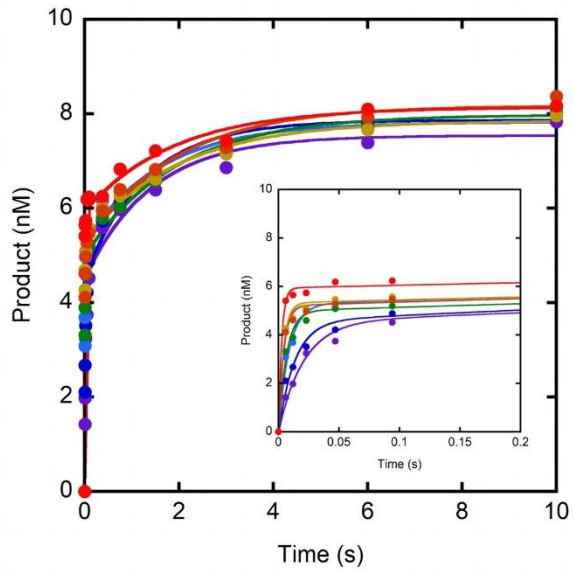
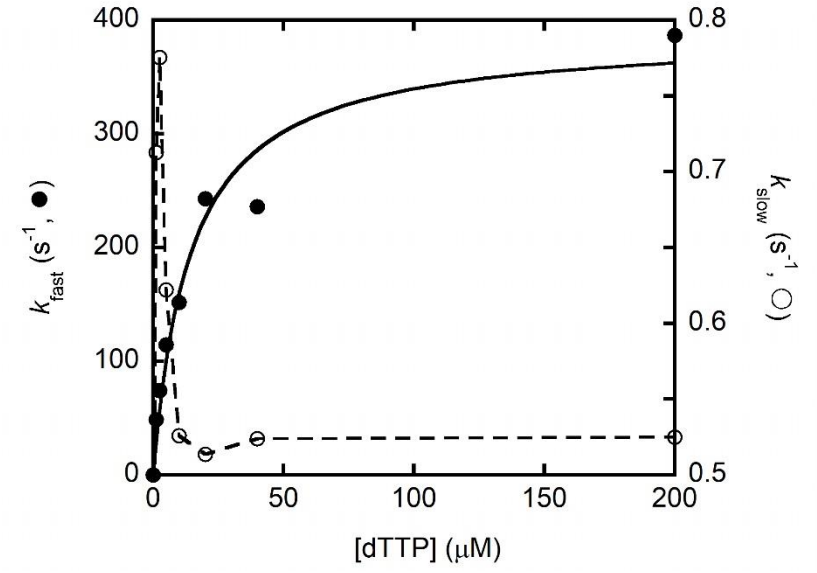
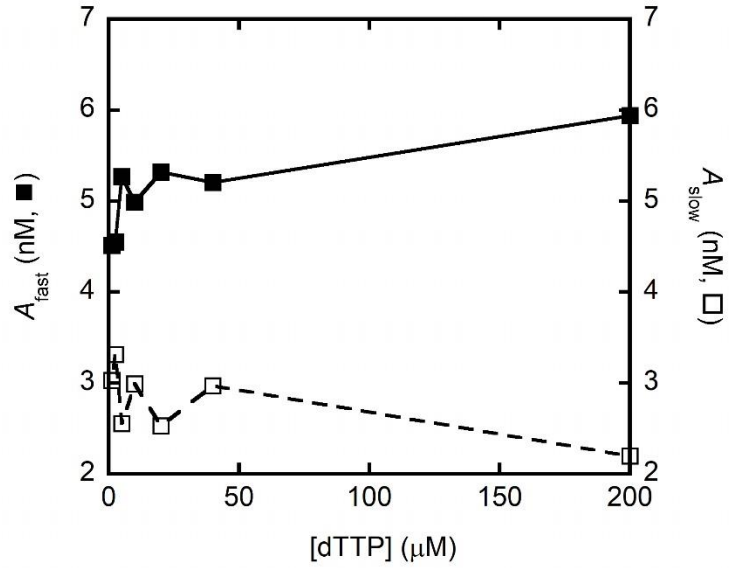
A


Product (nM)
Time (s)
0
2
4
6
8
10
Product (nM)
Time (s)
0
0.05
0.1
0.15
0.2

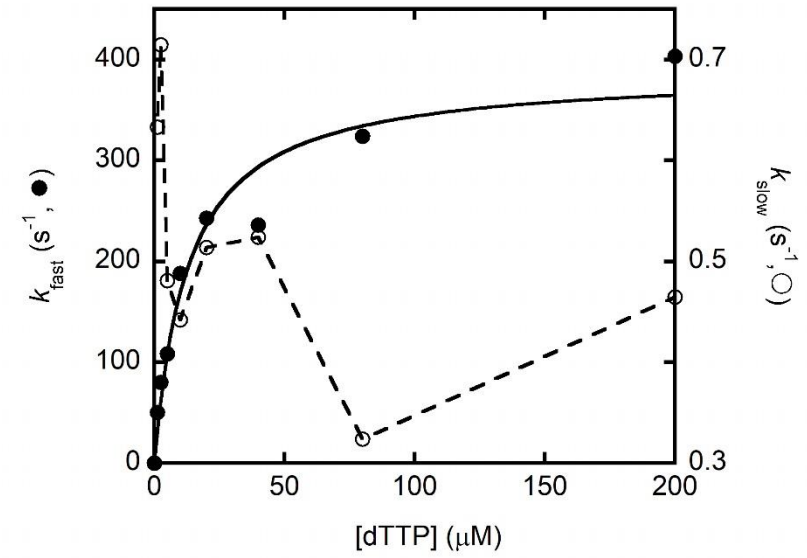
B


k_{fast} (s^{-1}, ●)
k_{slow} (s^{-1}, ○)
[dTTP] (μM)
0
100
200
300
400
0.5
0.6
0.7
0.8
0
50
100
150
200

C


7
6
5
4
3
2
0
50
100
150
200
[dTTP] (μM)
A_{fast} (nM, ■)
A_{slow} (nM, □)

D


400
300
200
100
0
0.7
0.5
0.3
0
50
100
150
200
[dTTP] (μM)
k_{fast} (s^{-1}, ●)
k_{slow} (s^{-1}, ○)

E

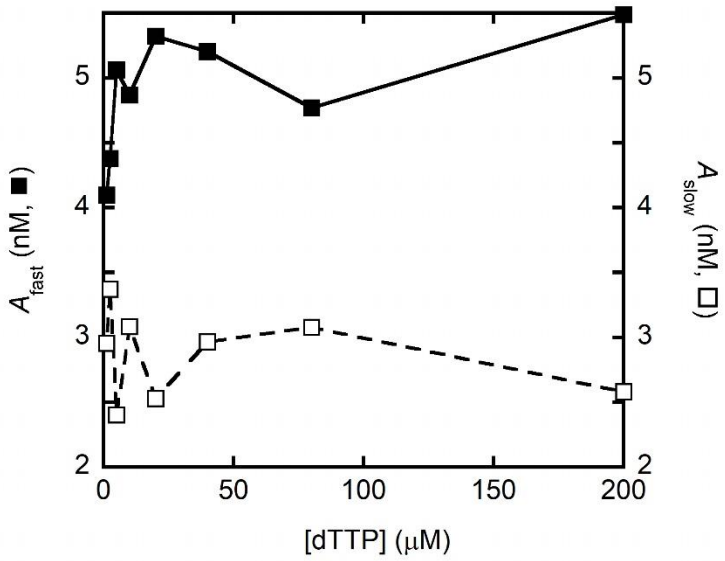


Figure S1. Replicate measurement of the pre-steady-state kinetics of correct nucleotide incorporation. (A) A pre-incubated solution of hPolε exo- (100 nM, UV concentration) and 5′-radiolabeled D-1 DNA substrate (20 nM) was rapidly mixed with 1.25 μM (purple), 2.5 μM (blue), 5 μM (light blue), 10 μM (green), 20 μM (yellow), 40 μM (orange) or 200 μM (red) dTTP and Mg^{2+} for varying incubation times before the reaction was quenched with the addition of EDTA. Product concentration was plotted against time and the data were fit to eq 5. (B) The k_{fast} and k_{slow} values for each dTTP concentration were plotted against their respective dTTP concentration. The plot of k_{fast} versus [dTTP] was fit to eq 7 to yield a k_{max} of 388 ± 30 s^{-1} and a K_d^{dTTP} of 14 ± 3 μM. The plot of k_{slow} versus [dTTP] was fit with a smoothed line. (C) The A_{fast} and A_{slow} values for each dTTP concentration were plotted against their respective dTTP concentration, and the data were fit with a smoothed line. (D) The average k_{fast} and k_{slow} values from the two replicates for each dTTP concentration were plotted against their respective dTTP concentration. The plot of k_{fast} versus [dTTP] was fit to eq 7 to yield a k_{max} of 388 ± 27 s^{-1} and a K_d^{dTTP} of 13 ± 3 μM. The plot of k_{slow} versus [dTTP] was fit with a smoothed line. (E) The average A_{fast} and A_{slow} values from the two replicates for each dTTP concentration were plotted against their respective dTTP concentration, and the data were fit with a smoothed line.

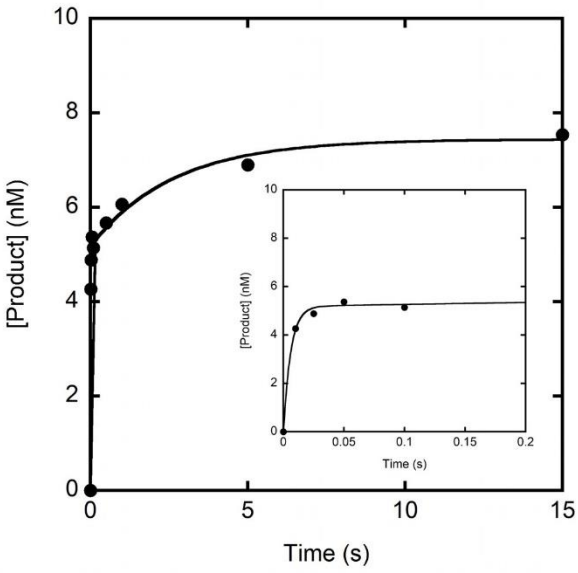


Figure S2. Pre-steady-state kinetics of correct nucleotide incorporation in the presence of a DNA trap. A pre-incubated solution of hPolε exo- (100 nM, UV concentration) and 5′-radiolabeled D-1 DNA substrate (20 nM) was rapidly mixed with 100 μM dTTP, 2 μM unlabeled D-1 DNA trap, and Mg^{2+} for varying incubation times before the reaction was quenched with the addition of EDTA. Product concentration was plotted against time and the data were fit to eq 5. The k_{fast} and k_{slow} values were 167 ± 23 s^{-1} and 0.4 ± 0.1 s^{-1}, respectively.

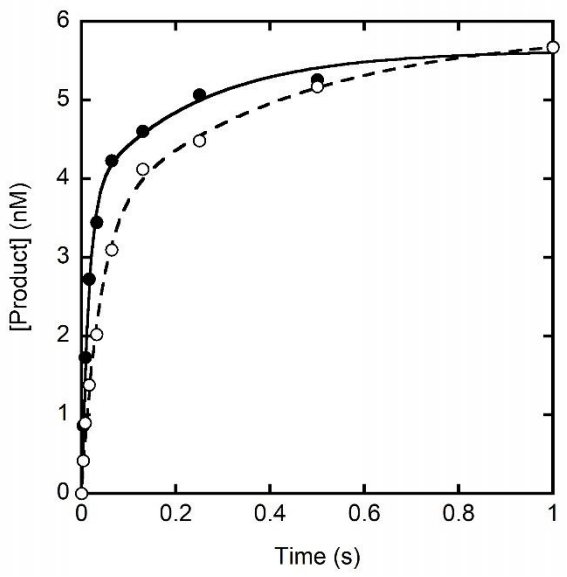


Figure S3. Comparison of correct nucleotide incorporation kinetics when using an EDTA or an acid quench. A pre-incubated solution of hPolε exo- (100 nM, UV concentration) and 5′-radiolabeled D-1 DNA substrate (20 nM) was rapidly mixed with 2.5 μM dTTP and Mg^{2+} for varying incubation times before the reaction was quenched with the addition of either EDTA (●) or 1 M HCl (○). Samples quenched with 1 M HCl were first extracted by phenol and chloroform and then were neutralized with the addition of 1 M NaOH. Product concentration was plotted against time and the data were fit to eq 5. The k_{fast} and k_{slow} values are about 2-fold higher when the reaction is quenched with EDTA than with 1 M HCl.