SUPPORTING INFORMATION

The Effect of A and B Metal Ion Site Occupancy on Conformational Changes in an RB69 DNA Polymerase Ternary Complex

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Calculation of kinetic parameters for Scheme 1.

Supplemental Figs. 1- 4

Supplemental material

In order to explain the kinetic parameters for Scheme 1 that we obtained from simulations, we calculated the value of K_{dg}^{app} that could not be determined directly from the observed or extrapolated values for k_{+3} , k_{-3} , k_{+4} and K_{dq}^{app} .

Scheme 1. The expanded kinetic scheme for primer extension.

In the minimal kinetic scheme for primer extension (Scheme 2), we know the following: $K_{eq} = K_1 \cdot K_2$; $K_1 = k_{+1}/k_{-1}$, $K_2 = k_{+2}/k_{-2}$; $K_{dq}^{app} = 1/K_{eq}$; $K_{dg}^{app} = 1/(K_1 \cdot K_2)$; $1/K_1 = K_{dq}^{app} \cdot K_2 = K_{dq}^{app} \cdot (k_{+2}/k_{-2}) = 150 \mu\text{M} \cdot (1000 \text{ s}^{-1}/4 \text{ s}^{-1}) = 37.5 \text{ mM}$. K_{dq}^{app} (150 μM) was determined from the chemical quench. k_{+2} ($\sim 1000 \text{ s}^{-1}$) was estimated from the k_{max} for the conformational change rate in the stopped-flow fluorescence experiments with increasing $[\text{Rh} \cdot \text{dTTP}]$ (Fig. 8) or $[\text{dTTP}]$ (Supp. Fig. 3) in the presence of 2mM CaCl_2 . k_{-2} (4 s^{-1}) was obtained from the pulse-chase-chemical-quench experiments (40). Therefore, from the calculation based upon the observed values, K_{dg}^{app} is equal to 37.5mM.

Scheme 2. The minimum kinetic scheme for primer extension.

We also can calculate the K_{dg}^{app} value from the kinetic parameters for Scheme 1 estimated from the values of the simulation results, where $K_{dg}^{app} = 1/K_2 = k_{-2}/k_{+2} = 4 \times 10^6 \text{ s}^{-1} / 100 \text{ s}^{-1} \mu\text{M}^{-1} = 40 \text{ mM}$. The above calculations suggest that our estimated parameters (40mM) generated using the KinTekSim software program are quite consistent with the values (37.5mM) we have obtained from the observation of other experiments.

Given a ground state K_{dg}^{app} for the nucleotide binding (40mM), k_{+3} of 2000 s^{-1} , and k_{-3} of 4 s^{-1} , the apparent K_{dq}^{app} of the nucleotide binding is $80 \mu\text{M}$, which is approximate the value that we see. In other words, we have observed a $k_{+3} > 1000 \text{ s}^{-1}$, a k_{-3} of 4 s^{-1} and an apparent K_{dq}^{app} of $150 \mu\text{M}$. Given these values, the ground state K_{dg}^{app} must be about 40mM. There is an overwhelming force driving the ground state to the closed conformation, (the 500 fold difference between k_{+3} and k_{-3} in addition to irreversible chemistry). Effectively, the [EDN] is close to zero. There is only free nucleotide and nucleotide bound in the closed conformation (FDN). In the absence of chemistry, only 1/500 of the enzyme bound fraction of the nucleotide would be in the EDN state. This is only true however for the correct dNMP incorporation. For incorrect dNMP incorporation, the driving force is greatly reduced because k_{+2}/k_{-2} is much smaller.

Figure Legends

Supplemental Fig. 1: Equilibrium fluorescence titrations of 1 μ M RB69 pol

complexed with 200nM ddP/T with increasing [Rh•dTTP]. The change in fluorescence signal versus [Rh•dTTP] fit a hyperbolic equation. For the **ddP/T** sequence see Fig. 2, A1. **(A)** With the A metal site empty, titration with Rh•dTTP gave a K_{dg}^{app} of $63.4 (\pm 4.9) \mu\text{M}$; **(B)** Titration with Rh•dTTP in the presence of 2mM Ca^{2+} , gave a K_{dg}^{app} of $9.0 (\pm 0.6) \mu\text{M}$. The fluorescence change (ΔF) with increasing [Rh•dTTP] indicates the extent of fluorescence quenching for the ternary RB69 pol:ddP/T:RhdtTP complex compared with the binary RB69 pol:dp/T complex.

Supplemental Fig. 2: Stopped-flow fluorescence scans of RB69 pol:ddP/T with

increasing [Rh•dTTP]. **(A)** One syringe contained 800nM **ddP/T** (for the **ddP/T** sequence see Fig. 2, A1) and $4\mu\text{M}$ RB69 pol in 50mM MOPS buffer, pH 7 at 24°C . The other syringe contained varying [Rh•dTTP](0, 10, 20, 30, 40, 75, 100, 125, 150, 200 and $250\mu\text{M}$ from top to bottom) in same MOPS buffer. Both syringes contained 2mM CaCl_2 . The fluorescence signal versus time fit a double exponential equation. The scans are shown in different colors for different [Rh•dTTP] and the fitting curves are shown in black; **(B)** Rates of the first phase from the double exponential versus [Rh•dTTP] fit a hyperbolic equation with a k_{max} of $510 (\pm 34) \text{s}^{-1}$, a K_{dr}^{app} of $43 (\pm 14) \mu\text{M}$ and Y-intercept of $8 (\pm 37) \text{s}^{-1}$. The average value of the rate of the second phase was $21.5 (\pm 6.4) \text{s}^{-1}$ and was independent of [Rh•dTTP]; **(C)** The amplitude change for the fluorescence quench versus [Rh•dTTP] fit a hyperbolic equation giving a K_{da}^{app} of $4.2 (\pm 1.1) \mu\text{M}$.

Supplemental Fig. 3: Stopped-flow fluorescence scans of RB69 pol:dp/T with

increasing [dTTP]. **(A)** One syringe contained 400nM **dp/T** (for the **dp/T** sequence see

Fig. 2, A2) and 2 μ M RB69 pol in 50mM MOPS buffer, pH 7 at 24°C. The other syringe contained varying [dTTP] (0, 2, 4, 5, 10, and 25 μ M from top to bottom) in same MOPS buffer. Both syringes contained 2mM CaCl₂ and 0.1mM EDTA. The fluorescence signal versus time fit a single exponential equation. The scans are shown in different colors for different [dTTP] and the fitting curves are shown in black; **(B)** The rate obtained from the fluorescence scans versus [dTTP] fit a hyperbolic equation with a $k_{\max}$ of 1200 ($\pm$ 50) s⁻¹, a K_{dr}^{app} of 6.8 ($\pm$ 0.6) μ M and Y-intercept of -10 ($\pm$ 24) s⁻¹; **(C)** The amplitude change for the fluorescence quench versus [dTTP] fit a hyperbolic equation with a K_{da}^{app} of 1.9 ($\pm$ 0.3) μ M.

Supplemental Fig. 4: Equilibrium fluorescence titrations of 1 μ M RB69 pol complexed with 200nM dP/T with increasing [dTTP]. For the dP/T sequence see Fig. 2, A2. The change in fluorescence signal vs [dTTP] fit a hyperbolic equation with a K_{dg}^{app} of 0.053 ($\pm$ 0.015) μ M. The fluorescence change (ΔF) with increasing [dTTP] indicates the extent of fluorescence quenching for the ternary RB69 pol:dP/T:dTTP complex compared with the binary RB69 pol:dP/T complex.

Supplemental Fig. 1

Supplemental Fig. 2

Supplemental Fig. 3

Supplemental Fig. 4