Supplemental Materials

Diverse Energetic Effects of Charge Reversal Mutations of Vaccinia Topoisomerse IB

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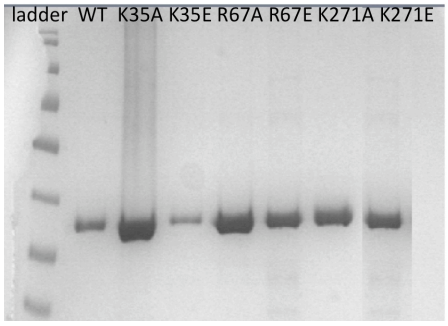

Figure S1: 12% SDS-PAGE gel of wild-type and mutant topoisomerases after expression and purification. The molecular weight markers are (bottom to top): 170, 130, 100, 70, 55, 40, 35, and 25 kDa. vTopo has a molecular weight of ~37kDa.

Grouping of Topoisomers into Pools and Validity of Kinetic Model. In order to simplify the kinetic analysis of supercoil unwinding, we grouped the 9 observed topoisomer bands into a substrate pool (S, bands 1-4), an intermediate pool (I, bands 5-8), and a product band (P, band 9). The goal of this procedure is to determine pool sizes where an individual topoisomer within the S and I pool would most likely relax to a new pool with a smaller linking number (i.e. S→I, S→P or I→P), rather than a different topoisomer within the same pool. To determine the S pool size, we simply measured the individual band intensities of the initial distribution of topoisomers at zero time as a function of reaction time with topoisomerase. Bands that showed intensity decreases with time were deemed substrate because these bands must have relaxed into bands that migrate below the initial topoisomer distribution (i.e. either I or P). This behavior of the substrate pool allowed the assignment of a reasonable pool size for the intermediates of four, and the remaining material is accounted for in the single product band. In kinetic simulations (see

below), we found no evidence that the S or I pools reacted at different rates (i.e. the cleavage rate is unchanged between substrates of different superhelical density). In addition, these pool sizes are consistent with the previous conclusion that wild-type vTopo removes approximately 5 supercoils per cleavage event in ensemble measurements with pUC19 DNA (*1*).

To demonstrate that grouping bands into the pools and employing the empirical kinetic model shown in Fig. 7a is valid, and also provides useful information about the processivity of these enzymes, we performed kinetic simulations using *DynaFit* (Fig. S2) (*2*). In the first simulation (Fig. S2A), which uses the optimized rate constants for wild-type vTopo, k_{cl} = 0.3, k_I = 5.0, and k_P =2.3 (see Fig 7a), a temporal increase in the intermediate pool and a lag in the product pool is observed. This indicates that most of the substrate pool must first go through the intermediate pool before being fully relaxed into product. However, if the ratio k_P/k_I is simply reversed from the 1/2 value observed with wild-type to 2/1 (i.e. k_{cl} = 0.3, k_I = 2.3, and k_P = 5.0.), then intermediates do not accumulate and more of the substrate goes straight to the product band (Fig. S2B). This second simulation mimics the k_{SP}/k_{SI} observed for the K271 mutant enzymes. Accordingly, the data K271A is well-fitted to using k_{cl} = 0.03 (due to the slower cleavage rate shown in Table 1), k_I = 0.26, and k_P = 0.53 so that the k_P/k_I = 2/1 (Fig. S2C).

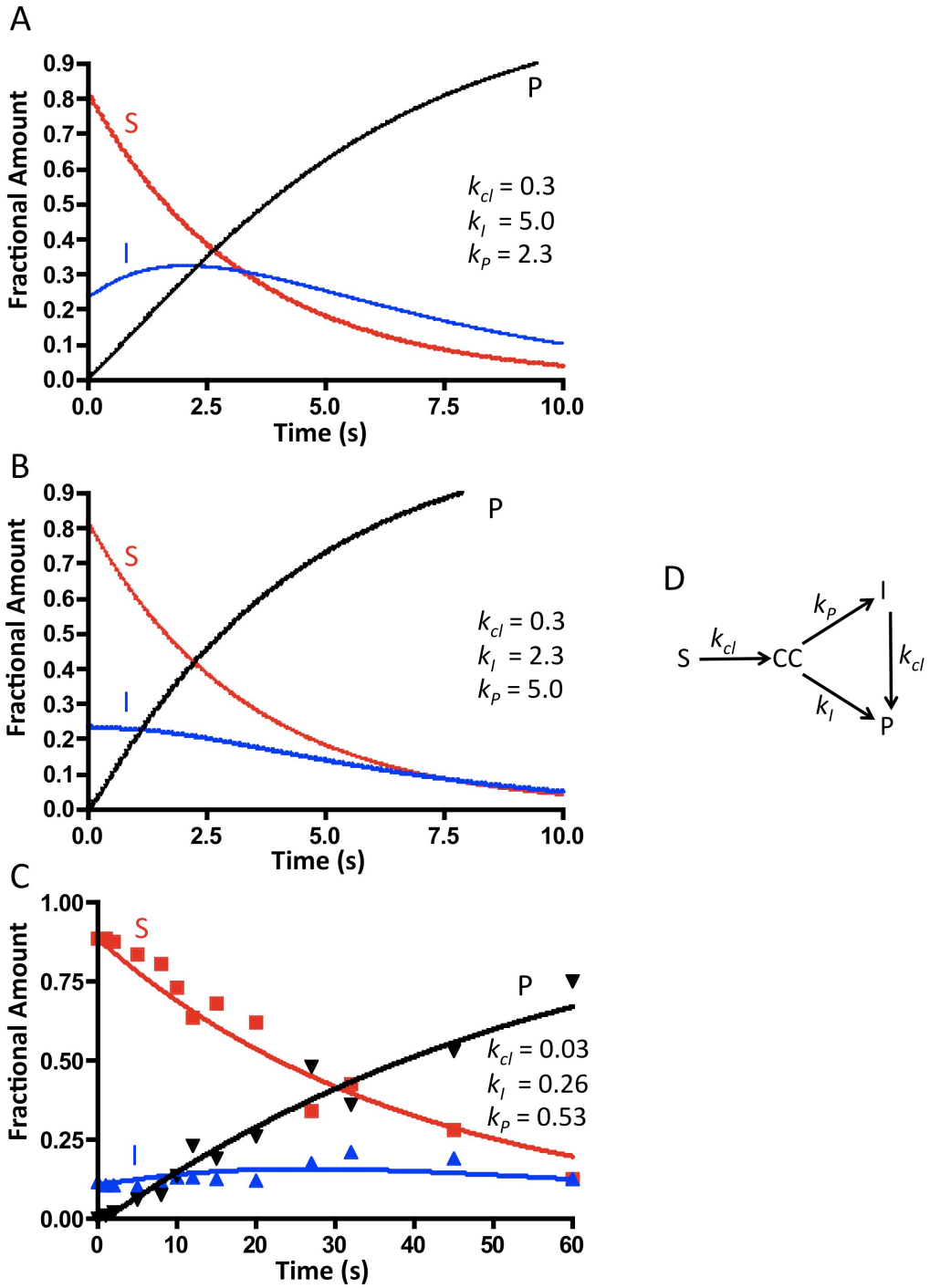

Figure S2 (A) Data simulation from DynaFit using the model shown in (D). Values used for k_{SI}, k_{IP}, and k_{SP} are shown. (B) DynaFit data simulation using the same model but with different kinetic parameters as shown to the right. (C) Timecourse of K271A supercoil unwinding that was fit using the values shown to the right in DynaFit. (D) Model used to create simulations for A-C. Abbreviation: S, substrate pool; CC, covalent complex; I, intermediate pool; P, product.

References

1. Stivers, J. T., Harris, T. K., and Mildvan, A. S. (1997) Vaccinia DNA Topoisomerase I: Evidence Supporting a Free Rotation Mechanism for DNA Supercoil Relaxation. *Biochemistry*. **36.** 5212-5222

2. Kuzmic, P. (1996) Program DYNAFIT for the Analysis of Enzyme Kinetic Data: Application to HIV Proteinase. - *Analytical Biochemistry. 237*, 260-73.