

SUPPORTING INFORMATION

Kinetic Mechanism for the Excision of Hypoxanthine by *Escherichia coli* AlkA and Evidence for Binding to DNA Ends

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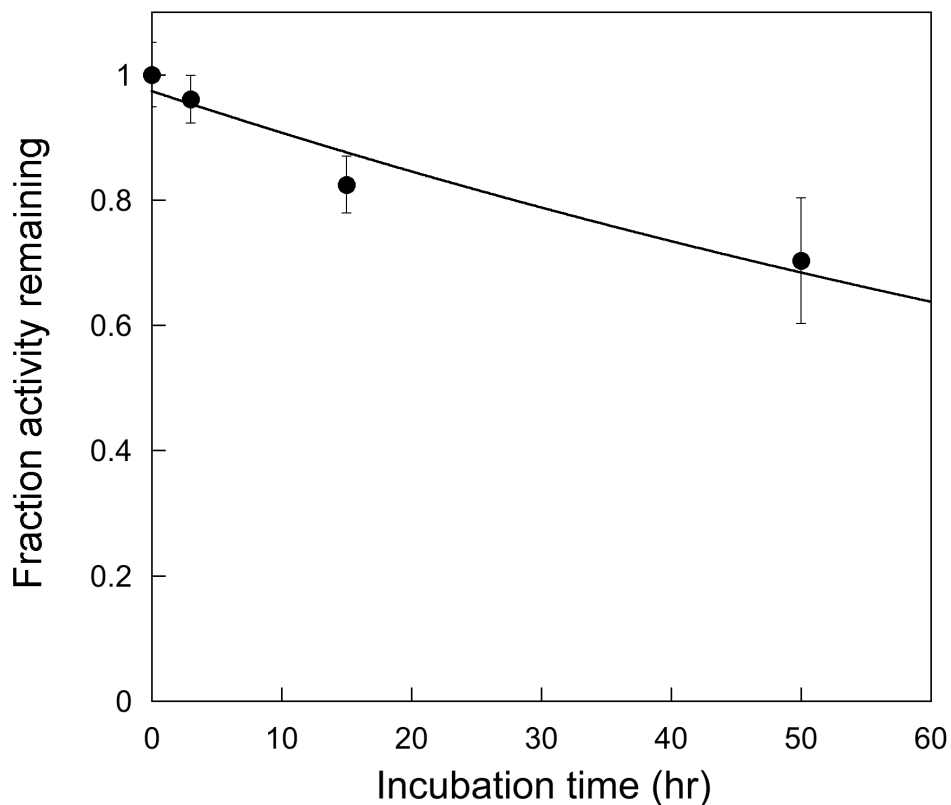


Figure S1. Stability of AlkA in the absence of DNA. AlkA (50 nM) was incubated for periods of up to 50 hours at pH 6.1 and 37°C. After the incubation period, multiple-turnover glycosylase activity was measured with saturating 19u I•T substrate (5 μ M). Reactions were performed in duplicate and the average and standard deviation is shown. The data were fit by a single exponential with a rate constant for inactivation of $0.007 \pm 0.001 \text{ hr}^{-1}$, which corresponds to a half-life of approximately 100 hours.

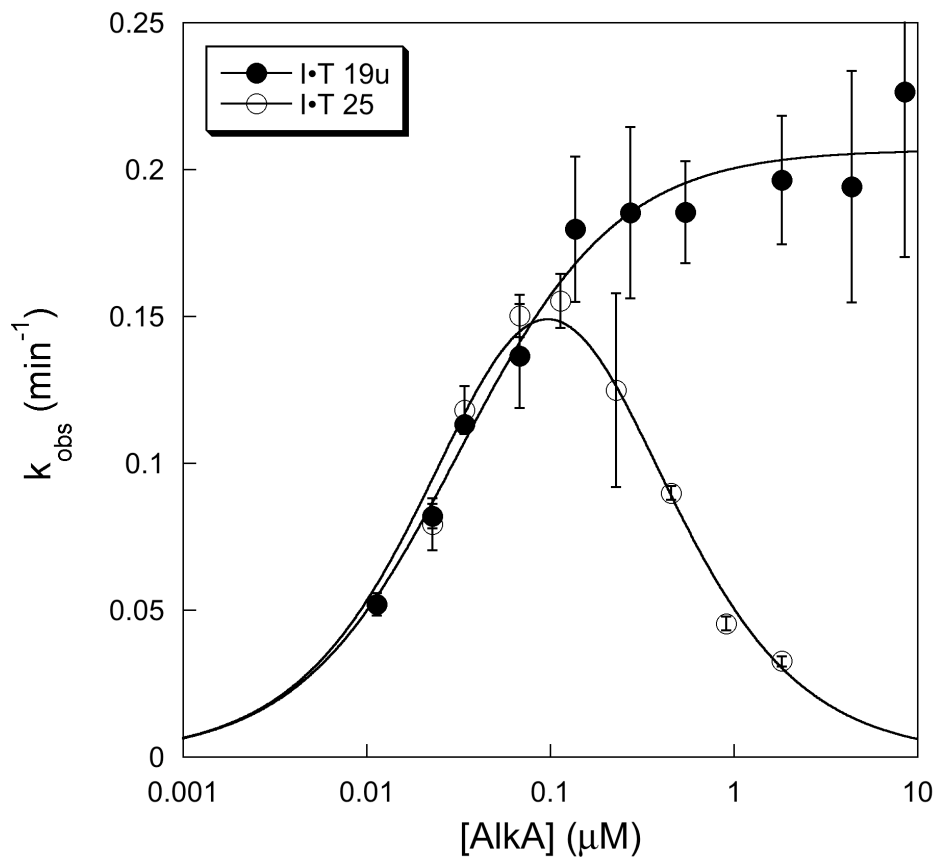


Figure S2. Single turnover excision of Hx by AlkA. The data for the 19u and 25mer substrates are shown from Figure 2B in the text as a semi-log plot to illustrate that the 19u substrate does not show any signs of inhibition up to 10 μM AlkA.

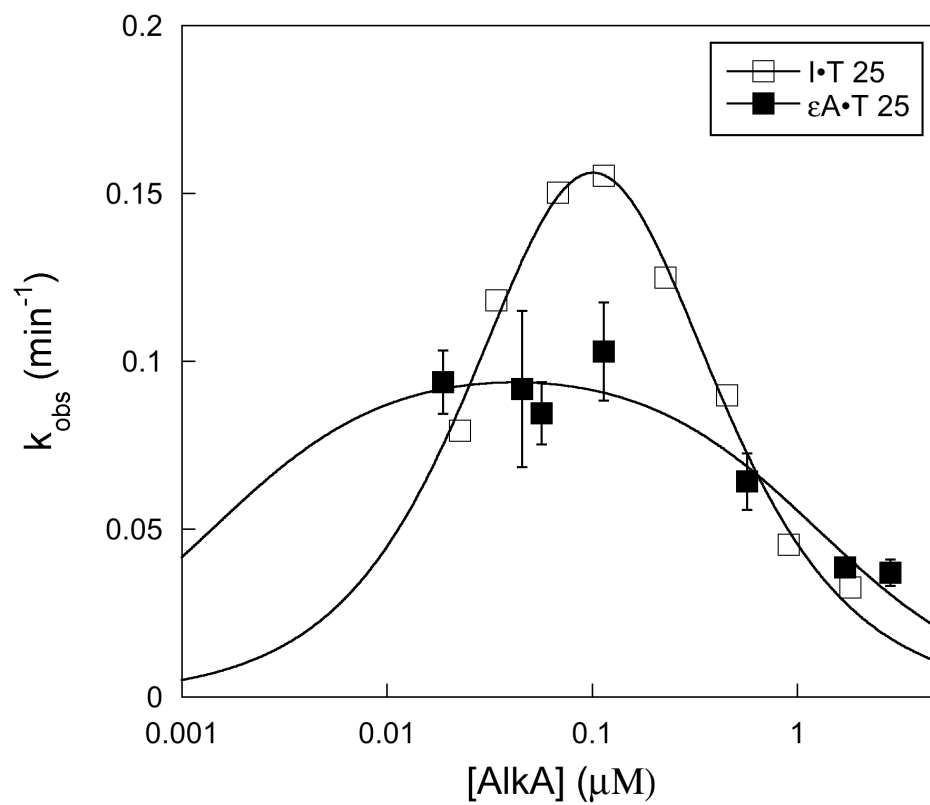


Figure S3. Single turnover excision of ϵA by AlkA. The 25mer $\epsilon A\bullet T$ substrate is identical to the 25mer $I\bullet T$ substrate, except the fluorescein label was attached to the 3' end (3'FAM). The data for the $I\bullet T$ substrate is from Figure 2 in the text.

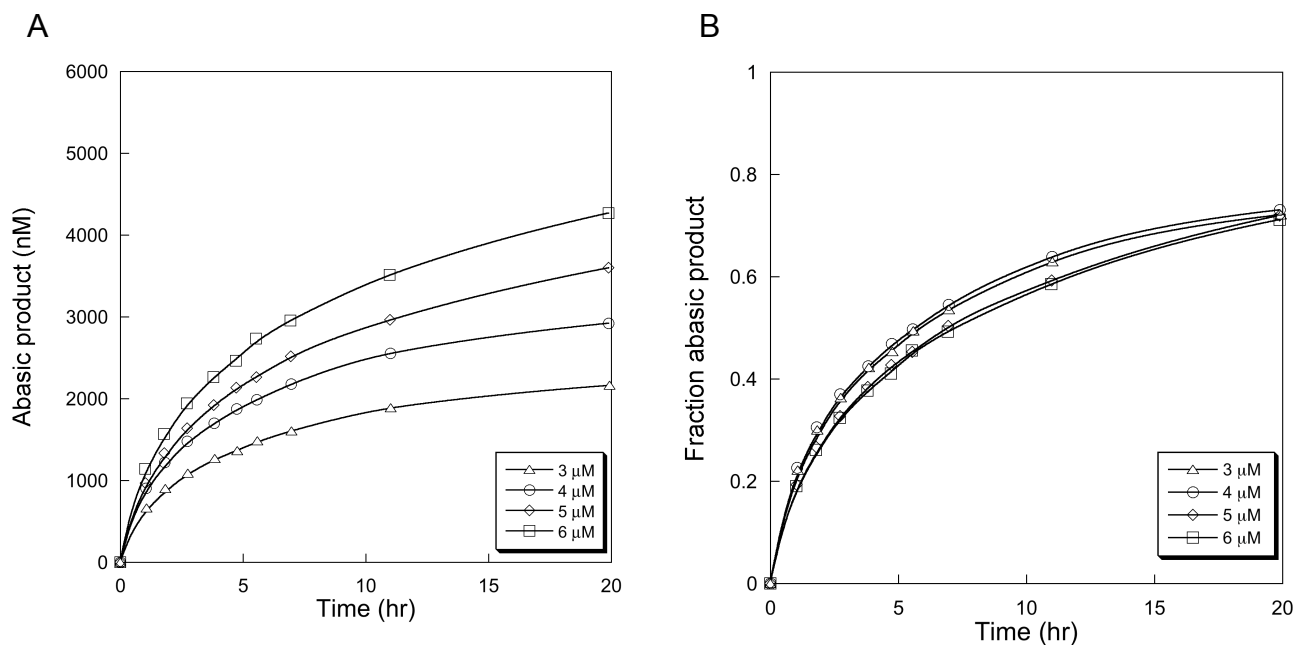


Figure S4. Evidence for product inhibition from long time courses. Representative reactions contained 10-fold excess of I•T 19u substrate over AlkA (see legend for substrate concentration). (A) Formation of abasic product shows significant curvature over time. Lines are not a theoretical fit to any equation, but simply drawn to facilitate comparison. (B) These data are also shown as the fraction of abasic product as a function of time. This illustrates that product inhibition is independent of the absolute concentration of substrate (or enzyme), but depends upon the ratio of product to substrate.