Supporting Information for

Human AP Endonuclease I Stimulates Multiple-Turnover Base Excision by Alkyladenine DNA Glycosylase

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Several additional supporting figures are provided. In Figure S1 the stability of Δ80 AAG is shown to be similar to that of full-length AAG under these conditions and BSA is required for full stability. The burst amplitude and steady state rate for turnover of the 25mer substrate by full-length AAG is shown in Figure S2. Figure S3 demonstrates that the single turnover excision of Hx is saturated at the concentrations of AAG employed at both 42 and 120 mM ionic strength. We show that the product complex formed in situ (i.e., in which both Hx base and abasic DNA products are present) also eliminates the burst phase (Figure S4). The observation of identical results obtained when both products are present (Figure S4) and when just the abasic DNA product is present (Figure 7 in the manuscript) suggests that Hx release is relatively rapid. Finally, we present a formamide quench control to show that the residual AP endonuclease activity under these conditions is ~10-fold slower than the glycosylase activity and therefore cannot be responsible for the stimulation of AAG (Figure S5).

Figure S1. Stability of Δ80 AAG was determined as described in the text for the full-length AAG (Figure 1). 20 nM Δ80 AAG was incubated at pH 7.0 and 37 °C. The ionic strength was adjusted with sodium chloride to be low (42 mM, circles) or high (120 mM, squares). Incubations contained no additional proteins (open symbols) or were supplemented with 0.1 mg/mL BSA (closed symbols). After the indicated incubation time the multiple-turnover glycosylase activity was measured as described in the Materials and Methods. The average for triplicate reactions is shown. In the absence of BSA there was no detectable glycosylase activity, so only the limit of ≤1% is indicated. Single exponential fits to the activity of AAG in the absence of BSA gave half-lives of ~40 minutes. In the presence of BSA full glycosylase activity was retained for at least a day.

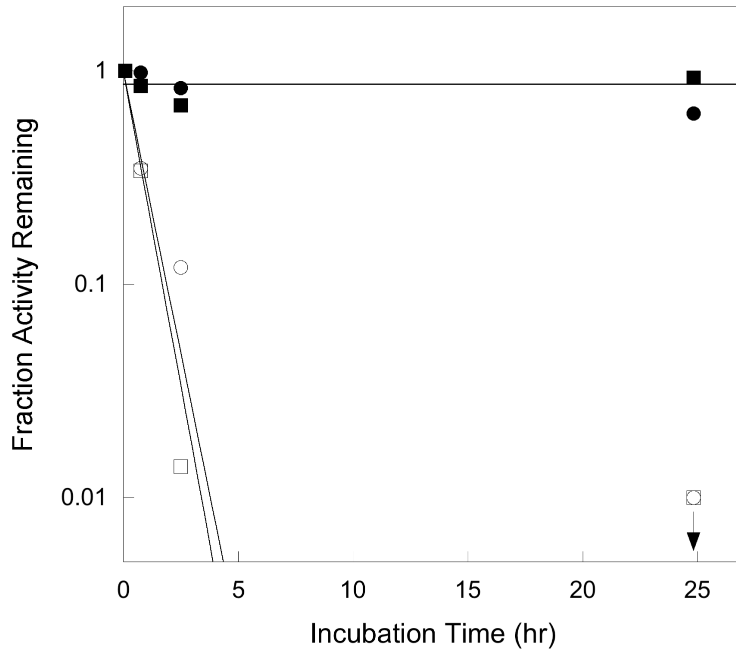

Figure S2. Burst kinetics for AAG-catalyzed excision of Hx. The burst amplitude and steady state velocity were determined as described in Figure 2 in the manuscript, but a lower range of AAG concentration was used. Both the burst amplitude (left axis; circle) and steady state velocity (right axis, square) are linearly proportional to the concentration of AAG. These data were obtained under the standard low ionic strength conditions with 1 μM 25mer substrate and the indicated concentration of full-length AAG. The error bars indicate the standard deviation from several independent reactions carried out under the same conditions.

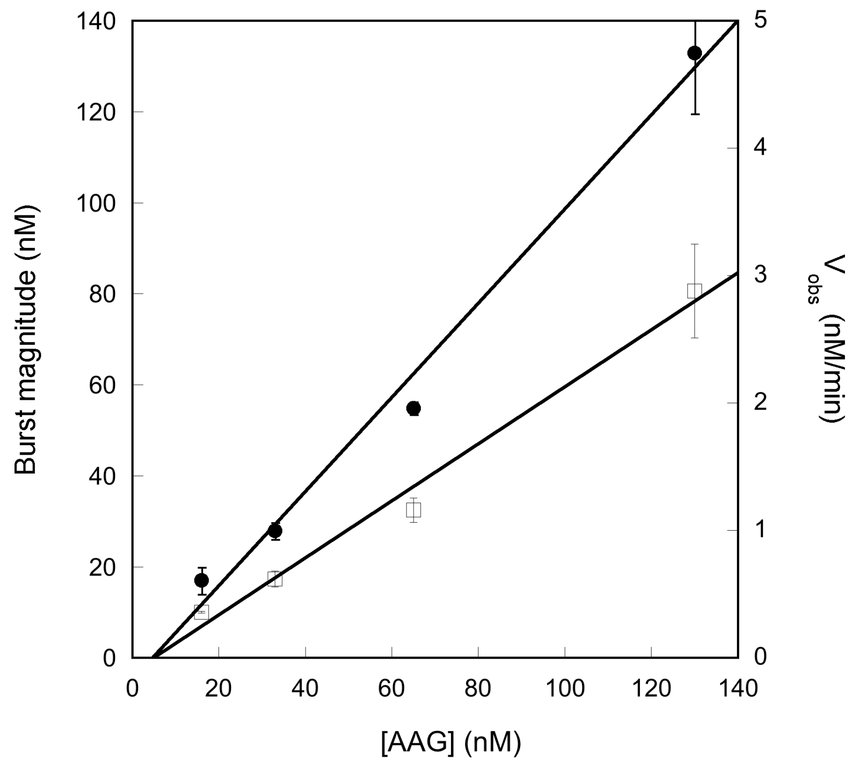

Figure S3. Single turnover excision of Hx by full-length AAG saturates at a k_{max} value of ~3 min^{-1} at both high and low ionic strength. The results from 42 mM ionic strength (closed circles) are compared to the results from 120 mM ionic strength (open circles).

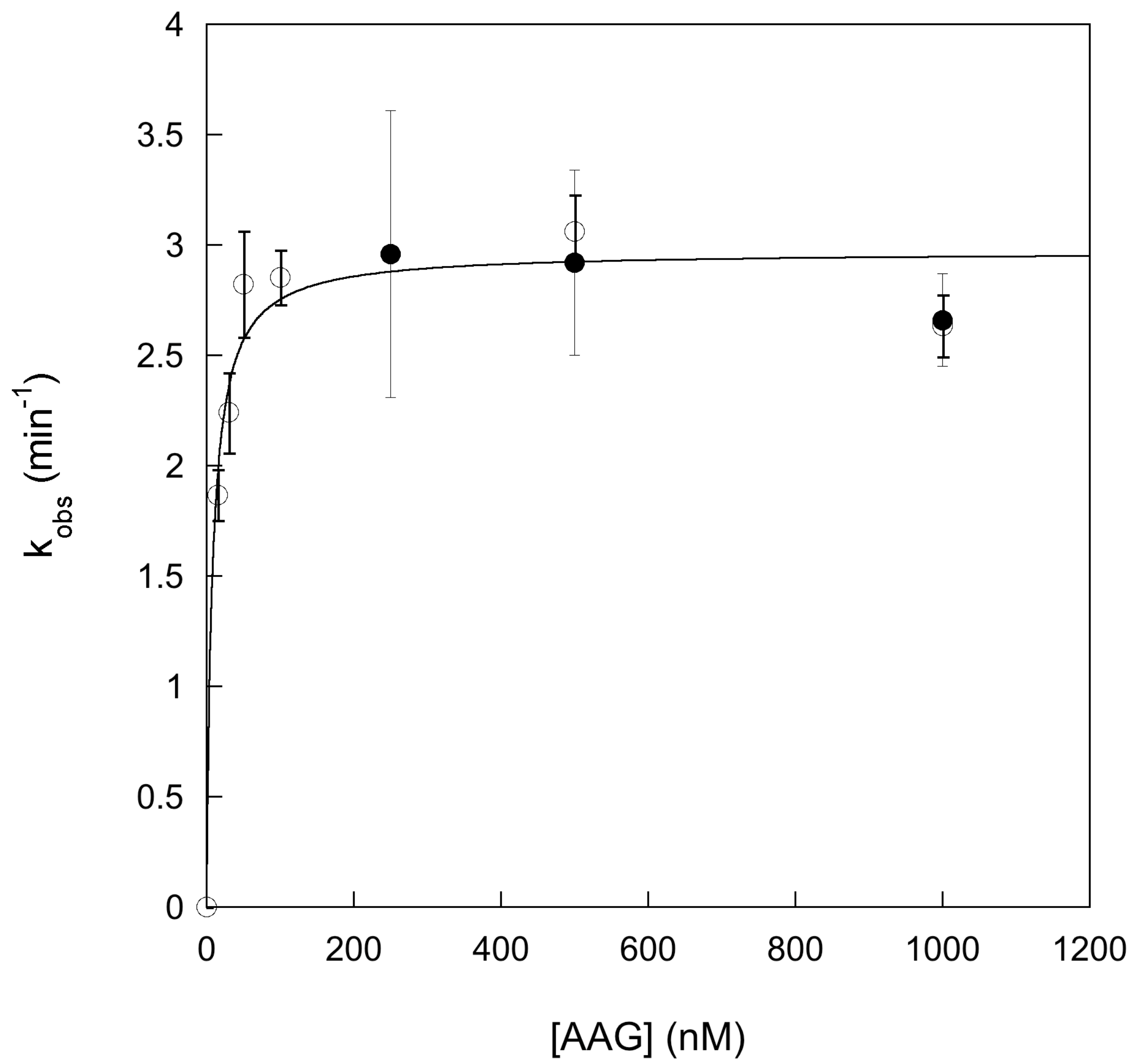

Figure S4. Burst kinetics result from rate-limiting product release. This experiment is analogous to Figure 7 in the manuscript, except that the AAG-bound product complex was formed in situ by allowing the single turnover reaction with one equivalent of substrate to go to completion before adding additional substrate.

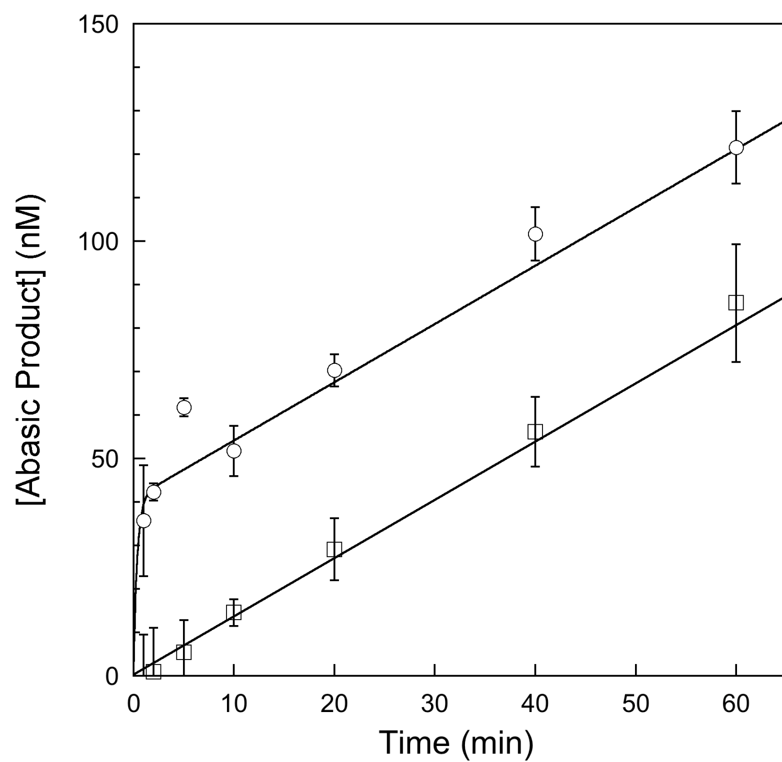

Figure S5. Control to show that the level of APE1-catalyzed DNA nicking does not account for the stimulation of AAG. The steady-state rate of formation of abasic sites by AAG (circles) is ~10-fold faster than the steady-state rate of formation of nicked DNA (squares). Under the standard low ionic strength (42 mM) conditions, 25mer substrate (1 μM) was incubated with 25 nM AAG and 2 μM APE1. Aliquots were taken out at the indicated times and quenched in 2 volumes of 0.3 M NaOH (circles) or with 5 volumes of 98% formamide/10 mM EDTA (squares). The hydroxide quenches were heated for 15 minutes at 70 ^{o}C to quantitatively convert any abasic sites into single strand breaks, then diluted with formamide/10 mM EDTA. All samples were heated for 3 minutes at 70 ^{o}C prior to running on a denaturing polyacrylamide gel and analyzing as described in the Materials and Methods. The results from two independent experiments were averaged and the error bars indicate the standard deviations. No correction was made for the ~10% spontaneous hydrolysis of abasic sites that occurred upon heating in formamide (data not shown), so the observed rate is an upper limit for the activity of APE1 under these conditions. This low level of activity is at least 10^6-fold lower than in the presence of Mg^{2+}. This control shows that no significant nicking occurs during the initial phase of the AAG-catalyzed reaction.