Isolating Contributions from Intersegmental Transfer to DNA Searching by Alkyladenine DNA Glycosylase

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Background: Human alkyladenine DNA glycosylase (AAG) uses facilitated diffusion to search the genome for sites of damage. Results: Biochemical assays demonstrate that AAG transfers directly between DNA molecules without macroscopic dissociation. Conclusion: Intersegmental transfer by AAG is rapid and does not require the flexible amino terminus. Significance: Efficient intersegmental transfer by this monomeric protein suggests that many proteins employ intersegmental transfer to search DNA.

Large genomes pose a challenge to DNA repair pathways because rare sites of damage must be efficiently located from among a vast excess of undamaged sites. Human alkyladenine DNA glycosylase (AAG) employs nonspecific DNA binding interactions and facilitated diffusion to conduct a highly redundant search of adjacent sites. This ensures that every site is searched, but could be a detriment if the protein is trapped in a local segment of DNA. Intersegmental transfer between DNA segments that are transiently in close proximity provides an elegant solution that balances global and local searching processes. It has been difficult to detect intersegmental transfer experimentally; therefore, we developed biochemical assays that allowed us to observe and measure the rates of intersegmental transfer by AAG. AAG has a flexible amino terminus that tunes its affinity for nonspecific DNA, but we find that it is not required for intersegmental transfer. As AAG has only a single DNA binding site, this argues against the bridging model for intersegmental transfer. The rates of intersegmental transfer are strongly dependent on the salt concentration, supporting a jumping mechanism that involves microscopic dissociation and capture by a proximal DNA site. As many DNA-binding proteins have only a single binding site, jumping may be a common mechanism for intersegmental transfer.

DNA repair proteins must search the entire genome to locate and repair rare sites of damage. Many of these proteins use nonspecific DNA binding that is mediated by electrostatic interactions to diffuse along the surface of the DNA (Fig. 1). This process, which is often referred to as facilitated diffusion, can occur by either hopping and/or sliding (1–3). In sliding, a protein maintains continuous contact with the phosphate backbone, effectively conducting a one-dimensional search. In hopping, a protein microscopically dissociates but rapidly reassociates on either the same or the opposing strand at a nearby site. These local sliding or hopping steps provide a highly redundant search that enables a DNA repair enzyme to detect sites of damage, but they are inefficient for covering large distances because diffusion is not directional (2). Therefore, it is advantageous to balance local search with long range transfer steps. Intersegmental transfer (i.e. direct transfer between DNA segments) is expected to be more efficient than random three-dimensional diffusion because it maximizes the time that the protein is actively searching DNA and minimizes the time that it is free in solution. Intersegmental transfer mechanisms have been invoked for a variety of different transcription factors (4–9), but few studies of DNA repair proteins have been reported. Recently, single molecule approaches were used to observe intersegmental transfer by a mismatch DNA repair protein, MutLα (10). The current work investigates whether an enzyme that initiates the base excision DNA repair pathway uses an intersegmental transfer mechanism to search DNA.

We examined the searching mechanism of human alkyladenine DNA glycosylase (AAG) (also known as MPG, methyl purine DNA glycosylase) because it has previously been shown to search DNA via facilitated diffusion (11). AAG is a small (33-kDa) monomer that is responsible for finding a wide variety of alkylated and deaminated purines in DNA, including 3-methyladenine, 7-methylguanine, 1,N4-ethenoadenosine (εA), hypoxanthine, and xanthine (12–15). This enzyme initiates the base excision repair pathway by catalyzing hydrolysis of the N-glycosidic bond to release the damaged base and create an abasic site. The structure of AAG in complex with εA-DNA has been determined (16), and stopped-flow experiments found

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3 The abbreviations used are: AAG, human alkyladenine DNA glycosylase; εA, 1,N4-ethenoadenosine; THF, tetrahydrofuran.
that AAG has pM affinity for this lesion (17). This tight binding makes εA an excellent substrate for studying diffusion because every recognition event results in N-glycosidic bond cleavage. The formation of an alkaline-labile abasic site provides an unambiguous chemical readout for encounter by a searching protein. Previously, AAG was found to make frequent hops to search both strands of DNA, and it is capable of diffusing past a tightly bound protein (18).

The AAG primary structure is highly conserved in vertebrates; however, the region of conservation is limited to the carboxyl-terminal region of ~220 amino acids. The amino-terminal region (80 amino acids in human AAG) is poorly conserved both in length and in amino acid sequence. This region of human AAG is proteolytically sensitive and appears to be flexible (19). Many eukaryotic DNA-binding and DNA repair proteins also have disordered amino or carboxyl termini (20–22), and it is proposed that one role of these flexible regions is to enable bridging interactions between two segments of DNA (23, 24).

We present a new biochemical assay to detect intersegmental transfer that is based upon tethering of DNA substrates with polyethylene glycol (PEG) linkers and measuring the frequency of correlated enzymatic action on the linked substrates. These assays and supporting kinetic characterization of intermolecular transfer rates demonstrate that AAG is capable of rapid intersegmental transfer. Extrapolation of the rates measured at low concentrations of DNA to the expected concentration of DNA in the nucleus indicates that both intermolecular and intramolecular diffusion occur on the same time scale. This suggests that AAG is able to balance an efficient local search with the global search for DNA damage. Although the amino terminus of AAG enhances DNA binding affinity and decreases the rate constant for dissociation, it is not required for intersegmental transfer. We conclude that this monomeric DNA-binding protein is capable of intersegmental transfer via microscopic dissociation and capture by a new DNA strand during a transient encounter, without the requirement for a bridging intermediate.
fixed substrate to enzyme ratio of 100:1. The initial rates were plotted versus the substrate concentration and were fit to the linear equation (\( \frac{V}{[E]} = k_{\text{off}} + k_{\text{trans}[\text{DNA}]} \)). The y-intercept of the fit represents the intrinsic dissociation rate of the enzyme from the abasic product, and the slope is the second order rate constant for direct transfer from the abasic product to a new substrate.

**RESULTS**

Processivity assays provide a powerful tool to quantify the ability of an enzyme to diffuse along DNA and to locate a specific site (27–29). With this approach, an initial binding event is monitored by enzymatic activity at one of two sites on a DNA molecule (Fig. 2A). Subsequently, partitioning between dissociation (\( k_{\text{off}} \)) and finding the second lesion (\( k_{\text{capture}} \)) can be determined. The excess of substrate over enzyme in the multiple turnover assays ensures that the probability of rebinding the same substrate molecule is negligible. The fraction processive (\( F_p \)) provides a quantitative measure of the macroscopic rate constants for dissociation and capture at the second site (\( F_p = k_{\text{capt}}/(k_{\text{capt}} + k_{\text{off}}) \)). This assay was previously used to establish that AAG conducts an efficient and highly redundant search of DNA (11, 18). In the current work, we covalently linked two duplexes using a flexible tether to test whether AAG is capable of direct transfer between duplexes. The purpose of the tether is to allow processivity assays to be used to correlate the enzymatic action on two tethered molecules. Second, the flexible linkage provides a high effective concentration of the two duplexes relative to each other that ensures that intersegmental transfer between the linked duplexes is favored over transfer between separate DNA molecules. For this assay to accurately report on intersegmental transfer, it is critical that the protein is not able to diffuse along the tether. In the following section, we investigate the possibility of using single-strand DNA or artificial PEG as a flexible tether. These studies reveal that single-strand DNA is not suitable because AAG is capable of facilitated diffusion on single-strand DNA. However, AAG is incapable of diffusing along PEG polymers, and these tethers could then be used to characterize the intersegmental transfer mechanism of AAG.

**Evaluation of the Diffusion of AAG on PEG and Single-strand DNA Polymers Using Direct Competition Assays**—It is known that AAG is strongly dependent on duplex DNA for its glyco-
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This is in contrast to conditions of very high salt (1000 mM NaCl) in which facilitated diffusion is not observed (Fig. 3C). All substrates were designed to have equivalent εA sites, but with different sizes to allow them to be analyzed simultaneously on the same denaturing acrylamide gels.

In each case, the indicated substrate was competed against the same 25-mer duplex substrate that served as a reference (Fig. 3A). Under conditions of 100 mM NaCl, the $k_{cat}/K_m$ values for the DNA with single-strand overhangs are ~2.5-fold larger than for the blunt end duplex, demonstrating that single-stranded DNA serves as a conduit for AAG to diffuse to the εA·T lesion site (Fig. 3D, open bars). In contrast, the PEG overhang has the same $k_{cat}/K_m$ value as the duplex, indicating that any binding of AAG to the PEG region does not result in productive transfer to the εA site. The substrate containing the PEG-10 tether to a single-strand extension shows a modest increase in $k_{cat}/K_m$ relative to the duplex with no extension, suggesting that the PEG linker acts as an insulator to AAG diffusion, but that AAG can transfer from the single-strand region to the duplex region directly (i.e. intersegmental transfer). As a control, we carried out the same set of competition experiments at 1 M NaCl. Under these conditions, the binding of AAG is reversible, and there is no facilitated diffusion (Fig. 3C). All of the substrates showed the same $k_{cat}/K_m$ values under these conditions (Fig. 3D, filled bars), confirming that the concentrations of DNA were correct. We conclude that AAG is capable of diffusing along single-strand DNA, but cannot diffuse on PEG. Therefore, we used PEG linkers in assays to detect intersegmental transfer.

Multiple Turnover Processivity Assays to Monitor Intersegmental Transfer—To determine whether intersegmental transfer contributes to the search for DNA damage by AAG, two oligonucleotide duplexes that each contained an εA lesion site were tethered by a PEG linker (Fig. 2B). Correlated excision at the two sites of damage prior to macroscopic diffusion provides evidence for intersegmental transfer between duplexes. The processivity for these tethered oligonucleotides was compared with that of a continuous duplex substrate with two εA sites separated by 25 bp (47E2F2; Fig. 2B), which was previously characterized (11).

Multiple turnover processivity assays were performed under conditions of partial processivity (200 mM Na+) because this condition is most sensitive to any changes in processivity (11). The processivity values of AAG with PEG tethers of 1, 4, and 10 units are the same within error of the intact duplex substrate (Fig. 4A; $F_p = 0.6$). These results provide compelling evidence that AAG is capable of rapid intersegmental transfer, with the rate of transfer far exceeding the rate of macroscopic dissociation. The lack of a length dependence for the different length PEG tethers is not surprising because PEG is extremely flexible and has a short persistence length (31). Previous studies of the properties of PEG tethers provide estimates for the effective concentration of tethered duplex that a protein bound to one duplex would experience (32, 33). For the PEG-10 substrate (tethered by 60 ethylene glycol units), the effective concentration is estimated to be ~3 mM, which appears to be sufficiently high to support rapid transfer.
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To test whether intersegmental transfer is sensitive to the concentration of cations, we measured the steady state processivity of AAG with the PEG-10 substrate across a wide range of sodium concentration and compared it with the processivity of the continuous duplex substrate. Between 50 and 300 mM Na\(^+\), AAG efficiently transfers between the tethered duplexes and has the same probability of excising the lesion as if the lesions were separated by a continuous duplex (Fig. 4B). Furthermore, the steady state rates are indistinguishable for the two substrates (Fig. 4C). These results establish that intersegmental transfer is efficient across a wide range of salt concentration and that it is fast relative to dissociation from the abasic product. However, it is not possible to infer from these data whether the intersegmental transfer step might be sensitive to the salt concentration. This is because the rate of macroscopic dissociation is strongly dependent on the salt concentration and the intersegmental transfer rate could change significantly without becoming the rate-limiting step.

Transient Kinetic Analysis of Transfer on Processivity Substrates—To gain deeper insight into the rate of intersegmental transfer, we performed pre-steady state glycosylase experiments with the continuous duplex and PEG-tethered substrates. Under burst conditions, with a 10-fold excess of substrate over enzyme, the individual steps can be monitored to gain insight into steps that are faster than the overall steady state rate. For these processivity substrates, the formation and disappearance of the intermediate with a single εA lesion follow a branched, two-step irreversible pathway, in which the first step is base excision and the second step is either capture at the second site or dissociation (Fig. 5A). Under conditions for which DNA searching and/or intersegmental transfer are slower than the rate of base excision, the transient accumulation of the intermediate with a single εA lesion excited will be increased and the subsequent decrease to steady state levels will be slowed.

At 50 mM NaCl, a clean burst in substrate disappearance is observed for both the continuous duplex and the PEG-tethered substrates (Fig. 5B, squares). This confirms that the concentrations of enzyme and DNA were correctly determined. From the same time points, the concentration of the product bands and intermediate bands (containing one abasic site and one εA lesion site) were also calculated. It should be noted that AAG does not prefer either of the lesion sites and that both of the intermediate and product bands are observed in very similar intensity (18). Therefore, the sums of the two intermediate species and of the two product species were calculated and plotted. The reaction progress curve of the intermediate species is the most informative (Fig. 5B, circles). The equations governing the formation of the intermediate were derived according to Fig. 5A (see the supplemental material for the derivation of these equations). Fitting this equation to the data for the continuous duplex substrate (Fig. 5B, blue circles) reveals that the rate constants for formation and breakdown of the intermediate species are the same within error (Table 1). For the PEG-10 substrate (Fig. 5B, red circles), the rate constant for the formation of the intermediate was also identical within error (Table 1). However, a reproducible increase in the level of intermediate was observed that was accompanied by a slightly slower rate of breakdown of the intermediate (Fig. 5B; Table 1). This suggests that a new step, presumably intersegmental transfer, is partially rate-limiting and contributing to the rate of capture of the second site. Although identical results were obtained in multiple independent experiments, the magnitude of this effect is small, and it is difficult to exclude experimental error.

Intersegmental transfer is expected to be sensitive to the salt concentration (4, 34), and therefore, we lowered the salt concentration to 20 mM and repeated the transient kinetic experiment that was described above (Fig. 5C). Both substrates showed the expected burst of substrate disappearance, and the multiple turnover rate was greatly decreased due to slower dissociation from the abasic product (11, 35). Nevertheless, the rate constant for removal of the first εA lesion is the same as observed at 50 mM Na\(^+\). For the 47E2F2 substrate, a noticeable delay was observed corresponding to a rate constant for finding the second εA lesion of 0.14 min\(^{-1}\). In contrast, the PEG-10 substrate exhibited a significantly slower rate constant for capturing the second εA lesion of 0.07 min\(^{-1}\) (Table 1), resulting in greater transient accumulation of the intermediate with a single εA lesion (Fig. 5C). These data allow the rate constants for intramolecular transfer across 25 bp of duplex and intersegmental transfer between tethered duplexes to be calculated (Table 1). Remarkably, the intersegmental transfer between duplexes tethered by 60 ethylene glycol units is only 3-fold...
more slowly than the intramolecular transfer between sites 25 bp apart on a continuous duplex (20 mM salt condition; Table 1). It is not possible to measure rates of transfer at physiological salt concentration using this assay because the rates are much faster than base excision, but it is apparent that AAG transfer is accelerated by increased concentration of sodium ions. For the PEG-10 substrate, the observed transfer rate constant increased by 7-fold when the sodium concentration was increased from 20 to 50 mM (Table 1).

Evidence of Intersegmental Transfer Obtained by Examining the DNA Concentration Dependence—The observation of burst kinetics (Fig. 5) demonstrates that dissociation from the abasic product is rate-limiting under low salt conditions. Intersegmental transfer to a new DNA molecule would provide an alternative pathway to dissociation and result in an increased reaction velocity (Fig. 6A). We therefore measured multiple turnover glycosylase activity on the standard 47-mer processivity substrate at a range of DNA concentration (0.2–4 μM) and the rate of AAG at infinite dilution (kchem), which yields the off-rate of AAG at infinite dilution (y-intercept) and the bimolecular rate constant constant (slope). The processivity was also determined as a function of DNA concentration under the same conditions (Fig. 6C). At 50 and 100 mM NaCl, the processivity decreases with increasing concentration of DNA. This demonstrates that intersegmental transfer begins to compete with the pathway for finding the second lesion at this higher salt concentration and µM concentration of DNA. The unimolecular and bimolecular rate constants determined from the data in Fig. 6B are both strongly dependent upon the salt concentration (Table 2).

Intermolecular transfer can also be monitored by the addition of competitor DNA that does not contain sites of damage. Transfer to undamaged DNA allows for more rapid dissociation or transfer to a new substrate molecule. As predicted by this model, multiple turnover reactions of AAG were linearly accelerated by the addition of undamaged duplex (Fig. 7A, open symbols). The observation of a significant rate increase, without a decrease in processivity, shows that intersegmental transfer to the competitor DNA is slower than intramolecular searching, but faster than AAG dissociation when the concentration of NaCl is 50 or 100 mM. However, at 150 mM NaCl and high concentration of competitor DNA, the rate of transfer becomes competitive with the macroscopic rate constant for finding the

| TABLE 1 |
| Rate constants from pre-steady state kinetic analysis of two-lesion substrates |
| kchem | kaplt | ktrans |
| 50 mM Na⁺ |
| 47E2F2 | 0.23 ± 0.01 | 0.26 ± 0.02 | 3 ± 3 |
| PEG-10 | 0.27 ± 0.04 | 0.19 ± 0.03 | 0.7 ± 0.5 |
| 20 mM Na⁺ |
| 47E2F2 | 0.27 ± 0.04 | 0.14 ± 0.01 | 0.3 ± 0.1 |
| PEG-10 | 0.31 ± 0.02 | 0.07 ± 0.01 | 0.09 ± 0.01 |

* The kchem, values were determined from the pre-steady state burst for the disappearance of substrate (Fig. 5). Values for kcaplt were determined by fitting the burst of intermediate formation and breakdown with supplemental Equation S9. The values reflect the mean ± S.D. (more than three independent determinations).

* The rate constant for transfer was calculated from the values of ktrans and kcaplt according to the scheme in Fig. 5A, and the error was estimated by propagation of the S.D. of the individual rate constants.

* No detectable delay was observed, indicating that the transfer step is at least 10-fold faster than the base excision step (ktrans).
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FIGURE 6. Transfer to a new DNA molecule is promoted at higher DNA concentration. A, minimal kinetic mechanism for the AAG-catalyzed reaction on an oligonucleotide with two sites of damage. At dilute concentrations of DNA, the rate-limiting step is dissociation from the abasic product ($k_{\text{off}}$), but at higher concentrations of DNA, an intermolecular transfer step ($k_{\text{trans}}$) accelerates the overall rate of reaction. Multiple turnover processivity assays were performed at 50–150 mM Na$^+$ using the 47E2F2 substrate. B, reaction velocities (mean ± S.D., n ≥ 3) were fit by linear regression ($R^2$ ≥ 0.94). C, the fraction processive was calculated as described under “Experimental Procedures” and analyzed by linear regression. The 50 and 100 mM NaCl conditions did not show a significant slope (p > 0.01; GraphPad Prism). At 150 mM NaCl, a modest, yet statistically significant, slope was observed (p < 0.0001), indicating that intersegmental transfer begins to compete with finding the second lesion. This additional pathway for transfer is illustrated by the dashed lines in panel A.

TABLE 2

<table>
<thead>
<tr>
<th>[Na$^+$] (mM)</th>
<th>$k_{\text{off}}$ (s$^{-1}$)</th>
<th>$k_{\text{trans}}$ (s$^{-1}$)</th>
<th>Extrapolated $k_{\text{trans}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.1 × 10$^{-5}$</td>
<td>50</td>
<td>1 × 10$^{-24}$</td>
</tr>
<tr>
<td>100</td>
<td>2.3 × 10$^{-4}$</td>
<td>270</td>
<td>6 × 10$^{-2}$</td>
</tr>
<tr>
<td>150</td>
<td>7.0 × 10$^{-4}$</td>
<td>360</td>
<td>8 × 10$^{-2}$</td>
</tr>
</tbody>
</table>

- Rate constant for dissociation from the abasic product from the intercept in Fig. 6B.
- The rate constant for dissociation from nonspecific DNA is substantially faster, because nonspecific DNA stimulates multiple-turnover by AAG (Fig. 7A).
- Bimolecular rate constants for intersegmental transfer are from the slopes in Fig. 6B.
- The rate constant for intersegmental transfer in vivo was estimated by assuming that 10% of the genome is accessible (~10 mV DNA bp); the observed bimolecular rate constant was divided by the length of the substrate (47 bp) and multiplied by 0.01 mV bp.
- Transient kinetic approaches could not be used to measure transfer on the PEG substrates at higher cation concentration, because transfer is much faster than base excision.

As a control, we compared the kinetic effects of nonspecific 25-mer competitor to those of a DNA that contains a synthetic tetrahydrofuran (THF) abasic site. This site is a structural mimic of the abasic DNA product (Fig. 2B), and the rate of AAG dissociation is expected to be similar. We observe essentially no effect of the added THF-DNA on the rate (Fig. 7A, filled diamonds), providing further support for the model that the rate effect observed with substrate or nonspecific competitor DNA is due to intermolecular transfer. The rate of intermolecular transfer is the same for the THF-DNA as for undamaged DNA because the decreased processivity is identical within error for the experiments with the two different DNA molecules (Fig. 7B, diamonds). The use of competitor DNA provides important controls that rule out alternative models, such as effects due to added DNA or incomplete saturation of the enzyme. However, it is not desirable to obtain quantitative values for the intermolecular transfer frequency ($k_{\text{trans}}$) from these data. This is because the DNA that is added will also act as a competitive inhibitor with respect to substrate binding and the overall reaction rates are a combination of inhibition and enhanced turnover via the intermolecular transfer pathway. Therefore, it is preferable to use the experiments that vary substrate DNA to obtain values of $k_{\text{trans}}$ (Fig. 6).

Probing the Contribution of the Amino Terminus of AAG to Intersegmental Transfer—Eukaryotic DNA-binding proteins commonly have disordered tails that have net positive charge. It has been proposed that these basic tails enable efficient intersegmental transfer by allowing for transient formation of a bridging complex (23, 24, 36). AAG similarly has a positively charged amino terminus that decreases the rate of dissociation and thereby contributes to processive searching (11). Although the catalytic domain (80–298 in human AAG) is highly conserved among AAG homologs, the amino terminus is poorly conserved and varies widely in length (Fig. 8A). The rate of N-glycosyl bond cleavage for the truncated protein (Δ80) is identical to that of the full-length protein (37). Therefore, we tested to what extent the truncated protein that lacks the amino terminus is capable of intersegmental transfer. Processivity assays with the PEG-tethered substrates clearly show that Δ80 AAG is capable of efficient intersegmental transfer (Fig. 8B). We also examined the effect of DNA concentration on the observed rate of dissociation, as described for full-length AAG. The intersegmental transfer rate, which is given by the slope in the DNA concentration dependence, is almost identical for the full-length and truncated protein (Fig. 8C). Although truncation does not alter the rate constant for intersegmental transfer, the intercept is ~30-fold higher for Δ80 AAG than for full-length AAG, confirming that the amino terminus increases the binding affinity for the abasic DNA product (11). These results demonstrate that the positively charged amino terminus of AAG is not required for intersegmental transfer.

DISCUSSION

It has been suggested that both short range intramolecular and long range intersegmental searching steps are required for the efficient search of genomic DNA (1, 38–40), but there is little experimental information about the relative contributions of the two pathways, particularly the intersegmental pathway(s). In studying the searching mechanism of AAG, we have developed simple kinetic assays that allow for the quantitative...
dissection of intersegmental transfer pathways. Previous studies have examined DNA-binding proteins that are multimeric or have multiple putative DNA binding domains (4, 5, 10). In the current work, we focused on the ability of a small, monomeric enzyme to search DNA. Our results strongly favor efficient intermolecular transfer at a biologically relevant DNA concentration.

Two mechanisms for intersegmental transfer have been suggested that are distinct from simple three-dimensional diffusion (1, 41). In the first mechanism, there is an intermediate state in which the protein is simultaneously bound to two DNA sites. This mechanism has historically been called simply intersegmental transfer (1), but we refer to it as the bridging mechanism to avoid confusion with the fact that there is more than one mechanism for intermolecular/intersegmental transfer (Fig. 9). In the second mechanism, the protein microscopically dissociates (hops) and encounters a second site on a separate DNA molecule (or a distant site on the same DNA molecule) that happens to be close by. Microscopic dissociation...
Of the two models for intermolecular transfer (Fig. 9), our results are best explained by the jumping mechanism, which involves microscopic dissociation and reassociation. We consider it unlikely that AAG could employ a bridging mechanism because the positively charged amino terminus is not required for intersegmental transfer (Fig. 8) and there is not an obvious secondary binding site. Furthermore, it is expected that a bridging intermediate in which two segments of DNA are simultaneously involved in microscopic dissociation and reassociation, but a hop is defined as an intermolecular transfer step, and a jump is an intersegmental transfer step. In some cases, the observation that the observed dissociation rates are dependent upon the concentration of the acceptor DNA has been taken as evidence for the bridging model of intersegmental transfer (4–6). However, it is important to recognize that this observation can also be explained by a jumping mechanism, whereby a hop to a new DNA segment occurs much more quickly than microscopic dissociation. If another DNA segment is nearby, then association with the new DNA segment results in a jump. If no other segments of DNA are nearby, then these microscopic dissociation events are usually resolved by returning to the same DNA molecule and would be classified as a hop.

We find that the positively charged amino terminus of AAG does not contribute to intersegmental transfer; however, it clearly tunes the DNA binding affinity to balance the partitioning between facilitated diffusion and three-dimensional microscopic diffusion. It is intriguing that this region is poorly conserved even among closely related mammals and raises the possibility that cellular parameters such as protein abundance and genome size might require a different level of processivity. Alternatively, it may be that animals differ in the efficiency with which they are able to repair deaminated and alkylated DNA.

The rate of intersegmental transfer that we measured at μM concentrations of DNA is slow, relative to intramolecular searching, but intersegmental transfer and intramolecular transfer are predicted to occur on similar time scales at the mM concentration of DNA that is in the nucleus. This conclusion is supported by the observation that AAG shows very rapid transfer rates between PEG-tethered duplexes for which the tether increases the effective concentration into the mM range (Fig. 5). The ability of AAG to engage in intersegmental transfer optimizes the efficiency of the search for DNA damage by allowing a searching protein to escape a local DNA site. Electrostatic interactions are the dominant factor influencing the ability of AAG to perform both intramolecular hops (11, 18) and intersegmental transfer. As most DNA-binding proteins employ positively charged DNA binding sites, these searching mechanisms may apply more broadly to other proteins that search the genome, including other DNA repair and replication proteins, transcription factors, and DNA-modifying enzymes.

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