Effective molarity in a nucleic acid-controlled reaction

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Abstract

Positioning of reactive functional groups within a DNA duplex can enable chemical reactions that otherwise would not occur to an appreciable extent. However, few studies have quantitatively defined the extent to which the enforced proximity of reaction partners in duplex DNA favors chemical processes. Here, we measured substantial effective molarities (as high as 25 M) afforded by duplex DNA to a reaction involving interstrand cross-link formation between 2'-deoxyadenosine and a 2-deoxyribose abasic (Ap) site.

The idea that enforced proximity can increase reaction rates and equilibria is pervasive in cell biology, enzymology, and physical organic chemistry. The concept of "effective molarity" provides a quantitative measure of the extent to which the enforced proximity of reaction partners in duplex DNA favors chemical processes. Effective molarities have been measured for such processes. Along these lines, Taylor and Dervan measured an effective molarity of 0.8 M for the alkylation of a guanine residue in duplex DNA by a bromoacetamide unit tethered via a six-atom linker to a triple helix-forming oligonucleotide bound in the major groove. Cole and Pires reported an effective molarity 3.1 M for alkylation of a guanine residue by a bromoacetamide unit connected by a five-atom linker to a 4-thiouridine residue on the opposing strand of a DNA duplex. Beyond these two studies, there is little information addressing the important question: how large should we expect the kinetic and thermodynamic "payoffs" to be, when two reactive species are positioned within the structure of duplex DNA? Given that an assumption of increased effective molarity underlies the design of all nucleic acid-templated reactions it may be useful to gain additional quantitative information that speaks to this question.

In the work reported here, we measured the effective molarity afforded by duplex DNA to the reaction of 2'-deoxyadenosine with a 2-deoxyribose abasic (Ap) site. This reaction generates interstrand cross-links in 15–70% yields at 5'-ApT/5'-AA sequences in duplex DNA via reversible reaction of the N6-amino group of 2'-deoxyadenosine with the aldehyde residue of an Ap site (Scheme 1). The rate and yield of da–Ap cross-link formation in duplex DNA can be readily measured by denaturing polyacrylamide gel electrophoretic analysis, making this a good system for assessing effective molarities in a nucleic acid-controlled reaction.

We first set out to measure the rate and equilibrium constants associated with this cross-linking reaction in two different
sequences of duplex DNA (duplexes A and B, Fig. 1). Cross-linking in these duplexes occurs at the locations indicated in Figure 1.41

The Ap-containing, 5'-32P-labeled duplexes A and B were generated as described previously40–44 by the action of the enzyme uracil DNA glycosylase on the corresponding 2'-deoxyuridine-containing duplexes.45–47 Subsequent time-dependent conversion of the resulting Ap-containing duplexes to cross-linked DNA was monitored by the appearance of a characteristic17,40–44 slow-moving band on denaturing polyacrylamide gels (Fig. 1 and S1). This process can be described by a kinetic scheme where cross-link forms reversibly in competition with products derived from cleavage of the Ap site in the un-cross-linked duplex (Scheme 2b). The differential equations describing the change in concentrations of various species with respect to time were solved by changing the kinetic parameters to give minimum residuals between calculated and measured concentrations at the specified time points. The cross-linking reaction was analyzed at both pH 5 and 7 (Figs. 1 and S1–S4). We obtained apparent rate constants of 1.6 ± 0.2 × 10⁻⁶ s⁻¹ and 3.5 ± 0.2 × 10⁻⁶ s⁻¹ and equilibrium constants of 0.57 ± 0.16 and 2.2 ± 0.4 for cross-link formation in duplexes A and B, respectively, in Hepes buffer (50 mM, pH 7, containing 100 mM NaCl) at 37 °C.

We speculate that the band migrating between the cross-linked duplex and the unmodified Ap DNA on the denaturing gel shown in Figure 1 is a “low-molecular-weight” (LMW) cross-link that arises from strand cleavage at the Ap site48–50 to generate the 3'-4'-hydroxy-2-pentenal-5-phosphate (3'-dRP) cleavage product, followed by reaction of the C4,β-unsaturated aldehyde group with a nucleobase on the opposing strand (Scheme 2). Although this product remains to be characterized, the structure may be analogous to the low molecular weight cross-links derived from C4'-oxidized Ap sites described by Greenberg and co-workers.51 Most important in the context of this work, identical values were obtained for k1 and k2 until gel electrophoretic analysis. Lane 1 is a marker consisting of the Ap oligomer treated with piperidine to induce strand cleavage; lanes 2–14 show the cross-linking reaction at times 0, 1, 2, 4, 6, 8, 20, 28, 48, 72, 72, 96, 120, 144 and 168 h. The cross-link band (this is because processes leading to generation of the low molecular weight cross-link are relatively minor under the conditions of these experiments).

We next sought a bimolecular reaction whose rate and equilibrium constants could be compared with the DNA cross-linking data. For this purpose, we employed the reaction of 3,5-bis-O-methyl-2-deoxy-β-ribofuranose (5, Scheme 3)22,51 with 2'-deoxyadenosine (dA). This reaction generates 6, a nucleosidic analog of the DNA cross-link 4. Reverse-phase HPLC analysis of the reaction between various concentrations of 5 (0.2–1 M) and 2'-deoxyadenosine (10 mM) in Hepes buffer (50 mM, pH 7, containing 100 mM NaCl) at 37 °C revealed time- and concentration-dependent growth of the diastereomeric nucleoside “cross-links” 6 (Fig. 2). Nonlinear least squares fitting of the data gave a formation rate constant of 1.4 ± 0.2 × 10⁻⁷ M⁻¹ s⁻¹ and an equilibrium constant of 0.51 ± 0.01 M⁻¹ for this reaction (Fig. 3). The method of initial rates54,55 gave a similar result. It may be worth noting that, while equilibrium constants formally are unitless, it is common for chemists, biochemists, and chemical engineers to attach units as
needed to express the effects that changes in reactant concentration exert on a system.\textsuperscript{56} The formation of \( \textit{6} \) was faster when carried out at pH 5 (Na\(_2\)HPO\(_4\) (10.3 mM), citric acid (4.9 mM), containing NaCl (100 mM)). For example, at a 1 M concentration of \( \textit{5} \) the calculated second order rate constant for the reaction was \( 1.2 \times 10^7 \text{M}^{-1}\text{s}^{-1} \) at pH 7 and \( 3.8 \times 10^6 \text{M}^{-1}\text{s}^{-1} \) at pH 5.

Characterization of the dA–Ap cross-linking reaction both “inside” and “outside” of duplex DNA allows us to calculate the kinetic and thermodynamic (equilibrium) effective molarities created by positioning of the 2′-deoxyadenosine and 2-deoxyribose reaction partners within the double helix (Table 1). In the best case (duplex B), the calculated second order rate constant for the reaction was \( 1.2 \times 10^7 \text{M}^{-1}\text{s}^{-1} \) at pH 7 and \( 3.8 \times 10^6 \text{M}^{-1}\text{s}^{-1} \) at pH 5.

In conclusion, our work provides a rare quantitative description of how spatial confinement within duplex DNA can increase reaction rates and equilibria. The effective molarities measured in this

<table>
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<th>Species</th>
<th>( K_{eq} )</th>
<th>( k_f \times 10^7 )</th>
<th>( EM_{thermo} ) (M)</th>
<th>( EM_{kinetic} ) (M)</th>
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<tr>
<td>Sequence A</td>
<td>0.57 ± 0.16</td>
<td>16 ± 2 s(^{-1})</td>
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<tr>
<td>Sequence B</td>
<td>2.2 ± 0.4</td>
<td>35 ± 2 s(^{-1})</td>
<td>4.3</td>
<td>25</td>
</tr>
<tr>
<td>dA + 5</td>
<td>0.51 ± 0.01 M(^{-1})</td>
<td>1.4 ± 0.2 M(^{-1}) s(^{-1})</td>
<td>11.1</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 2. Formation of the nucleoside cross-link by reaction of \( \textit{5} \) with 2′-deoxyadenosine. (Panel a) HPLC analysis of the reaction between dA (10 mM) and compound \( \textit{5} \) (800 mM) to generate \( \textit{6} \) at 37 °C in HEPES (50 mM, pH 7) containing NaCl (100 mM). (Panel b) Time course for the formation of \( \textit{6} \) from the reaction of dA (10 mM) with \( \textit{5} \) (0.2–1.0 M) in HEPES buffer (50 mM, pH 7) containing NaCl (100 mM) at 37 °C. The dashed lines represent calculated values resulting from least squares regression analysis.

Figure 3. Graphical representation of the second-order rate constant, \( k_f \), for the formation of \( \textit{6} \) from dA and \( \textit{5} \). The value \( k_f \) is the pseudo-first-order forward rate constant calculated from least squares regression, where \( k_f = k_f(\textit{5}) \). The slope = \( 1.1 \times 10^7 \text{M}^{-1}\text{s}^{-1} \), \( R^2 = 0.996 \).
system are substantial, especially given that nucleic acid-controlled reactions usually are carried out at very low oligonucleotide concentrations where background bimolecular reaction rates are low.

Acknowledgements

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Supplementary data

Supplementary data (experimental materials and methods and kinetic fitting of gel electrophoretic data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.022.

References and notes