Enzyme-like Rate Acceleration in the DNA Minor Groove. Cyclopropylpyrroloindoles as Mechanism-Based Inactivators of DNA

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CC-1065 (**1**), a potent antitumor antibiotic discovered by Martin et al.,[1] is exquisitely complementary to the minor groove of DNA.[2] In addition to showing exceptionally high DNA-binding affinity,[1,3] **1** alkylates the N-3 atom of adenine bases (Scheme 1) with high specificity for certain AT-rich sequences.[4,5] A similar enzyme-like specificity has been observed for synthetic cyclopropylpyrroloindole (CPI) analogs[6] and for related natural products[7] and synthetic agents.[8] In further analogy to enzyme−substrate reactions, the covalent transformation of **1** and related compounds is reversible under appropriate conditions.[9−11] It has been suggested that these reactions are accelerated by duplex DNA.[11,12] To date, however, few direct kinetic studies have been reported.[13]

In contrast to **1**,[3] **2** and **3** have sufficient solubility in aqueous−organic solvent mixtures to permit kinetic measurements of their solvolytic and nucleophilic addition reactions at low pH.[14] They also have sufficient binding affinity to DNA, as well as alkylation specificity and biological potency,[6,12] to allow a meaningful comparison to **1**. Using spectroscopic methods, we have measured the rates of alkylation of synthetic adenine-containing duplex DNA polymers with **2** and **3** as a function of base pair concentration. Our data reflect an enormous rate acceleration of nucleophilic addition to the CPI moiety in the DNA minor groove, approaching rate accelerations at enzyme active sites.

Compound **2** is insoluble in 10% (v/v) dimethylacetamide (DMA)/40 mM Tris−trichloroacetate buffer, pH 7.2. However, in the presence of a large excess of [poly(dA-dT)]$_2$ the UV/vis spectrum (Figure 1a) was virtually indistinguishable from that of **2** dissolved in 50% (v/v) ethanol/water.[14] Thus DNA effectively "solubilized" **2**. The immediate appearance of a strong induced circular dichroic spectrum when **2** was mixed with the DNA polymer (supplementary material) also supported rapid noncovalent complexation of the drug. First-order decay (k_{obs} = 1.1 × 10^{-3} s^{-1}) of the long-wavelength UV band of **2** produced the spectrum of the DNA adduct (Figure 1b).[15] The k_{obs} for the reaction of **2** with poly(dA)-poly(dT), 2 × 10^{-2} s^{-1}, was more than an order of magnitude faster than that for [poly(dA-dT)]$_2$. For both polymers, k_{obs} was independent of the DNA concentration from 1 to 0.1 mM base pairs (supplementary material). This was consistent with the spectral indications that **2** was in each case totally bound as a precovalent complex at the initial observation.

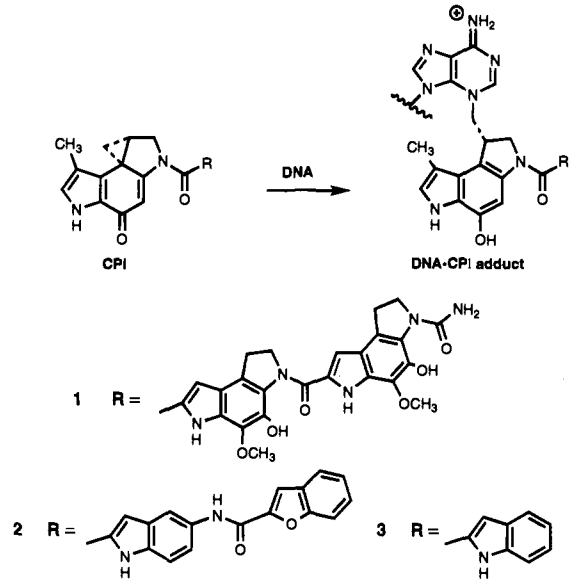
In contrast, k_{obs} for the reaction of **3** with poly(dA)-poly(dT) did show dependence on DNA concentration in this range (Figure 2). The data fit the Michaelis−Menten equation, with K_m = 0.4 mM, and k_a = 4 × 10^{-3} s^{-1}. These results are consistent with the kinetic model proposed for the reaction of CPIs with DNA, in which equilibrium formation of a precovalent complex is followed by a slower alkylation step.[6] Under these conditions of temperature and pH, the alkylation step can be considered irreversible.[9]

$$\text{CPI} + \text{DNA} \overset{1/K_m}{\rightleftharpoons} \text{complex} \xrightarrow{k_a} \text{adduct}$$

$$k_{obs} = k_a[\text{DNA}]/(K_m + [\text{DNA}])$$

Under conditions of saturation binding (>2 mM DNA, k_{obs} = k_a), the observed rate constant for the reaction of **3** with poly-

Scheme 1



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(13) The time course of the reaction of DNA with CPIs or related compounds has been followed by spectroscopy (ref 5) or HPLC (Asai, A.; Nagamura, S.; Saito, H. *J. Am. Chem. Soc.* **1994**, *116*, 4171−4177). An observed rate constant for the reaction of duocarmycin A with an oligonucleotide at one concentration has been reported (Sugiyama, H.; Hosoda, M.; Saito, I. *Tetrahedron Lett.* **1990**, *31*, 7197−7200).

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(15) Reactions were started by adding the CPI in DMA to solutions of the DNA polymer (0.1−2.5 mM base pairs) in 40 mM Tris−trichloroacetate, 1 mM Na_2EDTA buffer containing 10% (v/v) of DMA, final measured pH 7.0−7.2, at 25 °C. CPI concentrations ranged from 3 to 30 μM. Large excesses (base pairs:drug > 20:1) of DNA ensured pseudo-first-order behavior. Instrumental and data analysis methods were as described in ref 14. Reproducibility of observed rate constants was generally within 20%. Some variability reflected small differences in the final measured pH in individual experiments. At pH < 7, k_{obs} increased linearly with the hydrogen ion activity, with the slope dependent on ionic strength, the DNA polymer, and the drug (manuscript in preparation). Timed extractions of unreacted **2** from the reaction mixture, as well as changes in the DNA-induced circular dichroic spectra (supplementary material), verified the rate constants obtained by UV/vis. Thermal depurination of the spent reaction mixtures as previously described (ref 9) confirmed the essentially quantitative conversion of **2** to its N-3 adenine adduct. No solvolysis products were detected.

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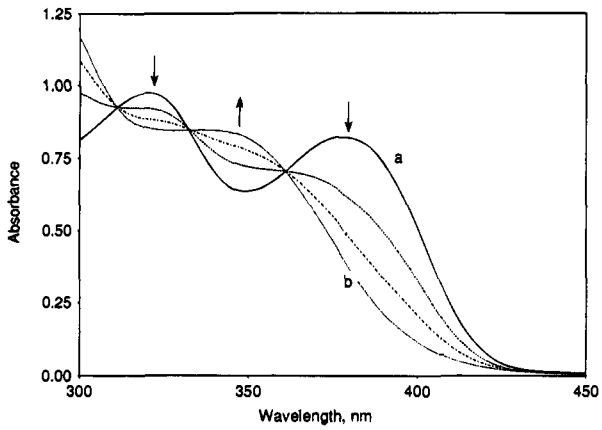

Figure 1. UV/vis spectra of 26 μM **2** and 1.2 mM base pairs of [poly(dA-dT)]$_2$ in 10% (v/v) DMA/40 mM Tris–trichloroacetate, 1 mM Na_2-EDTA, pH 7.2, 25 °C, (a) 1 min after mixing and (b) 90 min later. Intermediate curves shown at 10 and 20 min.

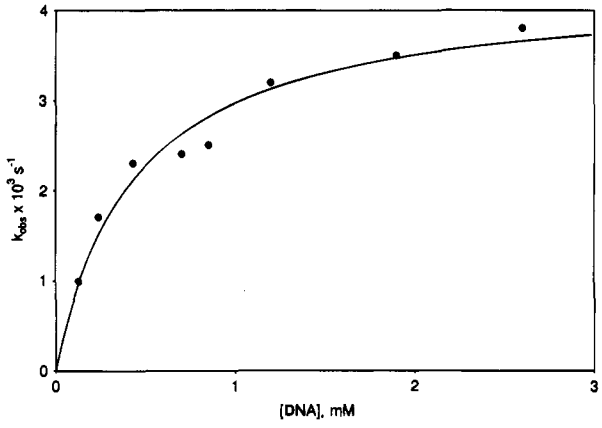

Figure 2. Observed first-order rate constants (ref 15) for the reaction of **3** with poly(dA)-poly (dT) as a function of base pair concentration. The curve was calculated from the Michaelis–Menten equation, with K_m = 0.4 mM and $k_a = 4 \times 10^{-3}$ s^{-1}.

(dA)-poly(dT) was only 5-fold lower than that for **2**. At 100 μM DNA, however, the difference in k_{obs} between **2** and **3** grew to 30-fold. These data illustrate that at sufficiently low DNA concentrations, precovalent binding affinity differences can have a profound effect on relative rates of alkylation. Thus, alkylation kinetics offers a plausible explanation for the 25-fold difference in cytotoxic potency reported for **2** and **3**.[6]

On the other hand, the difference in k_{obs} for the reaction of **2** with the alternating and homopolymers cannot be attributed to differences in K_m, since in both cases **2** was completely complexed to the DNA. These data indicate that k_a is sequence dependent, i.e., that dissimilar AT-rich minor groove environments can show significantly different stabilization of the transition state for CPI adduct formation. One component of this sequence difference might be the effective local proton concentration in the minor groove. Computational studies indicate an effective hydrogen ion concentration in the DNA minor groove that can be several orders of magnitude higher than in the bulk solvent.[16]

The formation of CPI–DNA adducts is formally analogous to the addition of neutral nucleophiles across the activated cyclopropyl ring of CPI. Hence, aqueous solvolysis of CPI is a reasonable standard reaction for rate comparison.[17] Extrapolation of CPI solvolytic data[14] to pH 7.2 gives an apparent second-order rate constant for addition of water to **2** or **3** of 2×10^{-10} M^{-1} s^{-1}.[18] For the reaction of **3** and poly(dA)-poly(dT) in 10% (v/v) DMA/water at this pH, the apparent second-order rate constant, k_a/K_m, is 8 M^{-1} s^{-1}. This is a rate difference of greater than 10^{10}! For **2**, k_a/K_m > 400 M^{-1} s^{-1}, indicating a rate difference of more than 10^{12}! Duplex DNA, therefore, can be as effective as many enzymes in accelerating chemical transformations. Furthermore, like enzymes, DNA appears to achieve this acceleration both by uniform binding (of the ground state and transition state) and by selective transition-state stabilization.[19] Because the DNA itself reacts with the CPI which it activates, to the detriment of its normal function,[20] the process is directly analogous to mechanism-based inactivation of enzymes.[21]

DNA catalysis of carcinogenic diol epoxide hydrolysis and platinum adduct formation has been reported,[22] and its acceleration of nitrogen mustard and nitrosourea alkylation has also been proposed.[23] However, the magnitudes of these effects are modest. The complex molecular architecture of **1** and related antibiotics, in contrast, has been honed by natural selection,[24] achieving an astounding degree of specificity and efficiency.

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Supplementary Material Available: The absorbance decay for the reaction of **2** with [poly(dA-dT)]$_2$ fit to a first-order rate equation, and to a four-parameter equation, the induced circular dichroic spectra for the reaction of **2** with this polymer, and a plot of k_{obs} vs base pair concentration for the reaction of **2** with both polymers (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(17) We have been unable to observe an addition reaction of adenine and U-71184 (a close analog of **2** available in greater supply). A solution of 0.4 mM U-71184 and 25 mM adenine in DMF was monitored at intervals by UV for 1 week at 45 °C and 3 additional weeks at 25 °C. Over this time period, the band at 353 nm decreased by about 30%, but this was virtually identical to the change observed in a solution of the CPI in DMF (no adenine) under these conditions. Granting that 10% nucleophilic reaction would be discernable, and neglecting the initial temperature elevation, an *upper limit* for the second-order rate constant for the reaction of adenine with U-71184 (and similarly with **2**), at 25 °C, may be estimated at 10^{-6} M^{-1} s^{-1}.

(18) From Table 2, ref 14, $k_{H^+} = 0.15$ M^{-1} s^{-1}, and thus $k_{H^+}[H^+]/[H_2O] = 2 \times 10^{-10}$ M^{-1} s^{-1}. These values were determined in 50% (v/v) ethanol/water; however, experiments with the electronically similar *N*-acetyl CPI showed only a small rate difference in this medium and in >99% water. This rate constant serves only as a point of reference, since the detailed mechanisms of the DNA and solution reactions may be different.

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