Kinetic Nature of Thiol Activation in DNA Cleavage by Calicheamicin

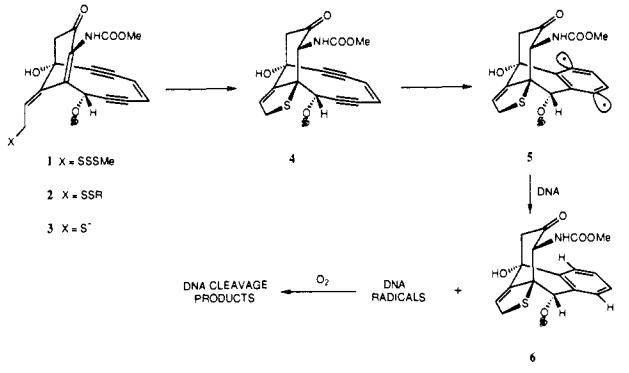
Moneesh Chatterjee, Katherine D. Cramer, and Craig A. Townsend*

Department of Chemistry
The Johns Hopkins University
Baltimore, Maryland 21218

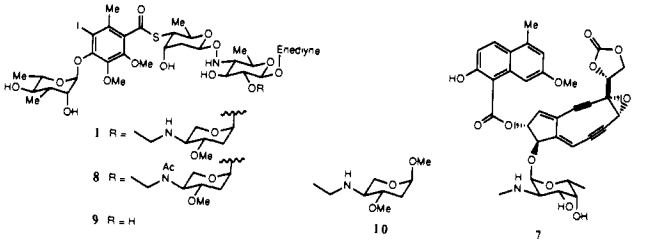
Received April 21, 1992

The activation of calicheamicin γ_1^1 for DNA cleavage is a complex process involving at least four steps (Scheme I). One of two kinetically significant events has been identified in the electrocyclization (Bergman rearrangement[1]) of dihydrothiophene **4** to the critical 1,4-diyl **5**.[2] In comparison, formation of the dihydrothiophene (**3** → **4**) and reaction of the 1,4-diyl with DNA (**5** → **6**) are much faster. However, it is the formation of allylic thiolate **3** that is the rate-determining step in the overall activation of the drug.[3] While the potential role of DNA itself to enhance the drug-induced cleavage process has been raised in another context,[2] recent observations in model reactions carried out in organic solvents have been taken to support the view that amino sugars present in calicheamicin (CLM γ_1^1, **1**) and the neocarzinostatin chromophore (NCS, **7**) may function as intramolecular bases in the DNA-bound forms of these drugs to deprotonate thiols and increase rates of drug activation.[4–6] To specifically address this issue, the reactions of CLM were examined under more physiologically-relevant conditions where the drug was largely bound to DNA.

Scheme I



If the proposed role of the amino sugars as intramolecular bases is correct, then **1** should demonstrate faster rates of reaction with thiols than analogues lacking the amino sugar. The first experiments monitored the reactions of CLM γ_1^1 (**1**), *N*-acetyl CLM γ_1^1 (**8**), and CLM α_3 (**9**) in the presence of aminoethanethiol by continuous UV assay.[7] The reaction of each of these compounds under pseudo-first-order conditions was found to be responsive to thiol concentration in keeping with the rate-determining role of this reaction in the overall activation process and, by varying thiol concentration, allowed their second-order rate constants to be accurately determined. The differences were slight and fell largely within the error limits of the method.[3]



To test experimentally whether DNA alters the reactivity of the allylic trisulfide of **1** to nucleophilic attack, solution kinetic analyses have been carried out using aminoethanethiol (AET) and glutathione (GSH) in the presence and absence of calf thymus DNA under pseudo-first-order conditions essentially as previously described[3] (70% Tris·HCl, NaCl/30% methanol, pH 7.50, 25.0 °C).[8] When **1** (10 μM) was treated with excess AET (1–10 mM), two sequential pseudo-first-order reactions were observed. Computer fitting of the data at each thiol concentration resolved the sulfur chemistry into the relatively fast formation of the anticipated mixed disulfide **2**,[5] followed by its slower decomposition to **3** (correlation coefficients >0.997). Monitoring the decrease in UV absorption at 315 nm could be used to measure both the formation of the disulfide **2**, owing to the unexpectedly lower extinction coefficient of the diynene at this wavelength, and the destruction of this chromophore on conversion to CLM ϵ (**6**). The rapid formation of the mixed disulfide was readily correlated by HPLC analysis. Replotting the observed pseudo-first-order rate constants as a function of thiol concentration gave second-order rate constants for reactions both in the presence and in the absence of DNA (Table I). These experiments were repeated with **1** (20–60 μM) and glutathione (7.5–40 mM). Higher concentrations of drug and thiol were used to compensate for slower reaction compared to the experiments above with AET (pK_a 8.3[9,10]) owing, at least in part, to the lower acidity of glutathione (pK_a 8.5[9]). Reactions of **1** to disulfide **2** and its subsequent conversion to CLM ϵ (**6**) were *slightly slower* in the presence of DNA for both thiols. This marginal decrease in rate owes, presumably, to steric retardation and possibly repulsion of thiolate approach by the polyanionic DNA helix harboring the bound drug.

While the DNA binding constants of **8** and **9**, compounds lacking a free amine, are not known[4] and, therefore, the >95% bound conditions may not be met, control experiments with AET showed no rate enhancements in the presence of DNA and at minimum demonstrate that the rate constant determinations are not adversely affected by the presence of DNA.

The pK_a of monosaccharide **10**[11] has been determined to be 8.2 ± 0.1 by NMR methods.[12] The amino sugar of CLM (**1**), therefore, is >85% protonated at physiological pH and, it may be argued, is more fully protonated in proximity to the negatively charged backbone of DNA. We have shown that binding to

(1) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25–31. Darby, N.; Kim, C. U.; Salaün, J. A.; Shelton, K. W.; Takada, S.; Masamune, S. *J. Chem. Soc., Chem. Commun.* **1971**, 1516–1517. Wong, H. N. C.; Sondheimer, F. *Tetrahedron Lett.* **1980**, *21*, 217–220, and references cited.

(2) De Voss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 4554–4556.

(3) Cramer, K. D.; Townsend, C. A. *Tetrahedron Lett.* **1991**, *32*, 4635–4638.

(4) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 697–699.

(5) Ellestad, G. A.; Hamann, P. R.; Zein, N.; Morton, G. O.; Siegel, M. M.; Patel, M.; Borders, D. B.; McGahren, W. J. *Tetrahedron Lett.* **1989**, 3033–3036.

(6) Myers, A. G.; Harrington, P. M.; Kwon, B-M. *J. Am. Chem. Soc.* **1992**, *114*, 1086–1087.

(7) The decrease in the absorbance of the diynene was observed at 315 nm, where DNA absorption was negligible.

(8) While CLM γ_1^1 can be solubilized by added DNA, it is comparatively insoluble in buffer alone. To obtain kinetic data for the drug in solution, the percent methanol required to achieve an 80 μM solution was monitored in a simple assay. CLM γ_1^1 (4 mM in methanol) was diluted with buffer and methanol as needed to make up fixed concentrations of the drug but varying proportions of methanol. These were assayed by HPLC against an internal standard. The suspensions/solutions were centrifuged at 16 000*g*, rt, 30 min, and the supernates were assayed as above and compared. Under these conditions the drug appeared completely dissolved at >26% methanol.

(9) Danehy, J. P.; Parameswaran, K. N. *J. Chem. Eng. Data* **1968**, *13*, 368–389.

(10) The pK_a was determined in 30% MeOH/70% H_2O by standard pH electrode titration: Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants*, 3rd ed.; Chapman and Hall: New York, 1984; Chapter 2.

(11) Kahne, D.; Yang, D.; Lee, M. D. *Tetrahedron Lett.* **1990**, *31*, 21–22.

(12) Markley, J. L. *Acc. Chem. Res.* **1975**, *8*, 70–80.

0002-7863/93/1515-3374$04.00/0

Table I. Bimolecular Rate Constants Determined for Reaction of CLM γ_1^I (**1**) with AET and GSH Measuring Its Reaction to Mixed Disulfide **2** and Conversion of the Latter to CLM ϵ (**6**)

	k (s^{-1} mM^{-1}) × 10^3		k (s^{-1} mM^{-1}) × 10^4	
	$\mathbf{1} \xrightarrow{-DNA} \mathbf{2}$	$\mathbf{1} \xrightarrow{+DNA} \mathbf{2}$	$\mathbf{2} \xrightarrow{-DNA} \mathbf{6}$	$\mathbf{2} \xrightarrow{+DNA} \mathbf{6}$
AET	8.31 ± 0.23[b]	5.29 ± 0.44	4.91 ± 0.16	2.55 ± 0.14
$t_{1/2}$ (min)[a]	1.4	2.2	23	45
GSH	6.90 ± 0.71	2.07 ± 0.27	1.04 ± 0.06	0.29 ± 0.03
$t_{1/2}$ (min)[a]	1.7	5.6	111	390

[a] Half-lives were determined in minutes assuming 1 mM thiol. [b] Errors are standard deviations from the slope upon a linear fit of the data.

DNA imposes no kinetic advantage on the amine with regard to thiolate activation of the drug. On the contrary, reaction is slightly slower in the presence of DNA, and the amino sugar is not a general-base catalyst.[3,13] While rate differences have been reported in organic solvents,[4,6] the amino sugars of CLM and, by inference, NCS play little role in drug activation in aqueous solution and simply enhance the affinity of each for DNA by establishing an energetically important ionic interaction with the sugar/phosphates of the duplex.[14] In support of this view, it is known that 100–1000-fold greater concentrations of **9** are needed to achieve the equivalent extent of DNA cutting shown by **1**.[4]

(13) Any possibility that the drug is not fully dissolved in the absence of DNA would only serve to increase the relative rate of solution reaction.

Acknowledgment. We thank Professor D. Kahne (Princeton) for providing a sample of amino sugar **10** and Drs. P. R. Hamann and G. A. Ellestad (Lederle) for samples of compounds **1**, **8**, and **9**. E. G. Casillas and S. M. McGuire are thanked for their help with the pK_a determination of **10** and the data transfer for analysis, respectively. We note extensive discussions with Professor A. G. Myers (Cal Tech) and regret that a mutual interpretation of the data could not be achieved. Financial support from the National Institutes of Health (CA 54421 to C.A.T. and GM 13525 to K.D.C.) and Lederle Laboratories is gratefully acknowledged.

Supplementary Material Available: Procedures for kinetic measurements and solubility determinations of CLM γ_1^I in aqueous methanol (3 pages). Ordering information is given on any current masthead page.

(14) The importance of analogous charge–charge interactions is well-recognized in protein–DNA interactions. See, for example: Woo, N. H.; Seeman, N. C.; Rich, A. *Biopolymers* **1979**, *18*, 539–552. See also: Ding, W-d.; Ellestad, G. A. *J. Am. Chem. Soc.* **1991**, *113*, 6617–6620, for the role of hydrophobic effects in the binding of calicheamicin to DNA.