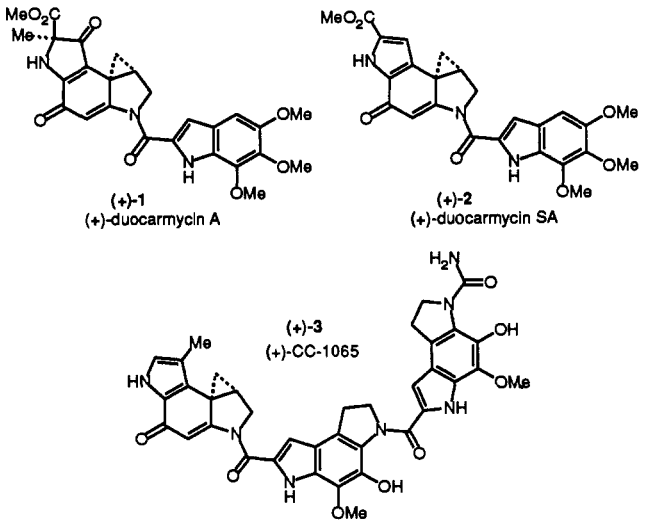
Reversibility of the Duocarmycin A and SA DNA Alkylation Reaction

Dale L. Boger* and Weiya Yun

Department of Chemistry, The Scripps Research Institute 10666 North Torrey Pines Road, La Jolla, California 92037

Received July 9, 1993

Duocarmycin A (**1**)[1] and duocarmycin SA (**2**)[2] constitute the parent agents of a growing class of potent antitumor antibiotics[3] that exert their biological effects through a sequence selective[4,5] minor groove alkylation of DNA.[6-11] To date, the duocarmycin A adenine N3 alkylation has proven similar, albeit subtly distinct, from that of (+)-CC-1065 (**3**)[7,9b,12-14] and structurally related analogs.[15-17] Herein, we detail the reversible nature of the (+)-duocarmycin A and SA[18] DNA alkylation reactions which complement the recent report[19] that simplified analogs of (+)-CC-1065, but not (+)-CC-1065 itself, reversibly alkylate duplex DNA. The ramifications of these observations and the distinctions in the relative reversibility of the reactions are discussed.



The detection of the reversible nature of the duocarmycin A or SA DNA alkylation reaction was conducted by following the transfer of agent from unlabeled duplex DNA covalently modified with **1** or **2** to unmodified singly 5′-end-labeled w794 duplex DNA.[7] The detection of the agent transfer reaction and the identification of the sites of labeled w794 DNA alkylation were established following thermolysis (100 °C, 30 min) of the covalently modified DNA, which induces thermal depurination and subsequent strand cleavage at the adenine N3 alkylation sites.[4,7,12] Unlabeled duplex w794 DNA was treated with **1** or **2** under conditions which ensure complete reaction of the agent. Unbound agent was removed using an extensive extraction protocol consisting of phenol extraction (1×), *n*-BuOH extraction (2×), and Et_2O extraction (1×) followed by EtOH precipitation and isolation of the DNA. This extensive extraction and precipitation procedure ensured that no unbound or noncovalently bound agent remained in the sample of unlabeled DNA. The covalently modified unlabeled DNA was redissolved in aqueous buffer, mixed with ^{32}P singly 5′-end-labeled w794 DNA at concentrations which represent 4:1, 1:1, and 0.5:1 molar ratios of unlabeled:labeled w794 DNA, and incubated at 50 °C (pH 8.4, 0.25 M NaH_2PO_4) for 8, 4, 2, and 1 day. Thermolysis (100 °C, 30 min), high-resolution PAGE, autoradiography, and quantitation of intact labeled DNA and the cleavage bands permitted detection of the alkylation of labeled w794 DNA, quantitation of the transferred agent constituting a measure of the reversible DNA reaction, and identification of the sites of labeled w794 DNA alkylation. The extent of the transfer of the agent and hence the reversibility of the DNA alkylation reaction proved sensitive to the incubation conditions. Increasing the pH (8.4 > 7.4 > 6.0), temperature (50 °C > 37 °C > 25 °C > 4 °C), time (8 days > 4 days > 2 days > 1 day), and to a lesser extent the salt concentration (200 mM NaCl ≃ 100 mM > 50 mM) led to increased transfer of agent from unlabeled to labeled duplex

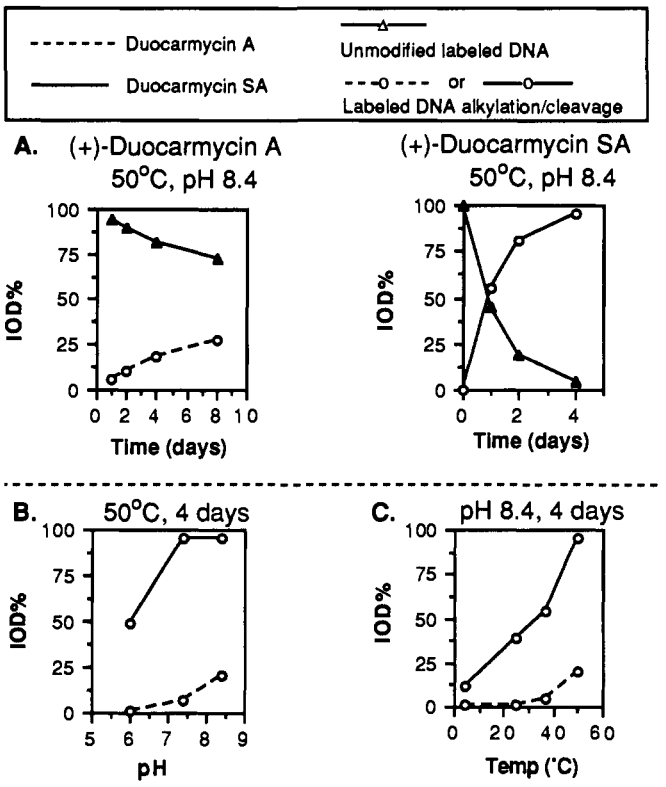


Figure 1. Time, pH, and temperature dependence of the reversible (+)-duocarmycin A (**1**) and (+)-duocarmycin SA (**2**) DNA alkylation reactions. The time (A), pH (B), and temperature (C) dependence was determined by incubation of 5′-end-labeled SV40 DNA (clone w794/w836) with **1**- or **2**-covalently modified unlabeled w794/w836 DNA (1:4 molar ratio) followed by thermally induced cleavage (100 °C, 30 min), 8% denaturing PAGE, and autoradiography. Integrated optical density (IOD) was determined for the unmodified labeled DNA (–▲–) and the alkylation site cleavage bands (duocarmycin A- - -o- - -; duocarmycin SA–o–) on a Bio Image Model 60S RFLP system and plotted against time (A, 50 °C, pH 8.4), pH (B, 50 °C, 4 days), and temperature (C, pH 8.4, 4 days).

(1) Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915.
(2) Ichimura, M.; Ogawa, T.; Katsumata, S.; Takahashi, K.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1991**, *44*, 1045.
(3) Yasuzawa, T.; Iida, T.; Muroi, K.; Ichimura, M.; Takahashi, K.; Sano, H. *Chem. Pharm. Bull.* **1988**, *36*, 3728.
(4) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961.
(5) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 4499.
(6) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645.
(7) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.
(8) Boger, D. L.; Yun, W.; Terashima, S.; Fukuda, Y.; Nakatani, K.; Kitos, P. A.; Jin, Q. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 759.
(9) Reviews: (a) Boger, D. L. *Chemtracts: Org. Chem.* **1991**, *4*, 329. (b) Boger, D. L. In *Advances in Heterocyclic Natural Products Synthesis*; Pearson, W. H., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 2, pp 1–188.
(10) Sugiyama, H.; Hosoda, M.; Saito, I.; Asai, A.; Saito, H. *Tetrahedron Lett.* **1990**, *31*, 7197.
(11) Yamamoto, K.; Sugiyama, H.; Kawanishi, S. *Biochemistry* **1993**, *32*, 1059. Sugiyama, H.; Ohmori, K.; Chan, K. L.; Hosoda, M.; Asai, A.; Saito, H.; Saito, I. *Tetrahedron Lett.* **1993**, *34*, 2179.

0002-7863/93/1515-9872$04.00/0

DNA, Figure 1. Notably, the extent and rate of reversibility (SA > A) parallels the relative reactivity of the two agents (A > SA)[20] and the more reactive agent provides the more stable, less reversible covalent adduct. In contrast to duocarmycin A and SA, but like the independent observations of Warpehoski et al.,[19] no reversible DNA alkylation reaction was observed with (+)-CC-1065. (+)-CC-1065 possesses an inherent reactivity which is less than that of (+)-duocarmycin A but greater than that of (+)-duocarmycin SA. The lack of observation of a reversible (+)-CC-1065 DNA alkylation reaction suggests that the ease or rate of reversibility is additionally dependent upon the extent of the noncovalent binding stabilization of the agent. Consistent with this interpretation, simplified analogs of CC-1065 do reversibly alkylate duplex DNA.[19]

The ramifications of these observations are especially significant. In the course of early studies on (+)-CC-1065, computational and quantitative molecular modeling studies in conjunction with an appreciation of the properties of the agents led us to propose that the alkylation reaction could be expected to represent a reversible, near thermal neutral reaction stabilized by extensive noncovalent binding interactions.[9b,21] The observation that the adenine N3 addition reaction does not occur with free adenine external to duplex DNA reinforced the view that the dominant forces driving the DNA alkylation reaction were not the covalent bonding forces but rather the noncovalent binding stabilization derived from hydrophobic binding and van der Waals contacts,[22] a process we continue to refer to as hydrophobic-binding-driven bonding.[9,21] That is, the inherent, readily reversible nature of the alkylation reaction is rendered less reversible or irreversible by dominant noncovalent binding interactions.[23]

The importance of these proposals has become apparent in the comparison of the biological properties of the agents. Like the comparisons of the biological properties of (+)-CC-1065 (**3**), (+)-CPI-PDE-I_1, and (+)-CPI-$CDPI_n$ (n = 1–3) with those of (+)-*N*-BOC-CPI,[14] the exceptionally potent cytotoxic activity of (+)-**2** (0.01–0.001 nM, L1210)[20] versus the relative nonpotent activity of (+)-*N*-BOC-DSA (6 nM, L1210)[20] may be attributed to the simple event of noncovalent binding stabilization of the inherently reversible DNA alkylation reaction.[14] Thus, the establishment of the slow, reversible nature of the duocarmycin DNA alkylation reaction, the establishment of the reversible CPI DNA alkylation,[19] and their dependence on the relative reactivity of the alkylation subunit as well as the extent of the noncovalent binding affinity confirm earlier proposals and suggest that this feature of the agents' behavior may play a fundamental role in the expression of their biological properties.[24]

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA55276 and 42056).

Supplementary Material Available: PAGE figure illustrating the Figure 1A experimental data (1 page). Ordering information is given on any current masthead page.

(12) Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315.
(13) Hurley, L. H.; Warpehoski, M. A.; Lee, C.-S.; McGovren, J. P.; Scahill, T. A.; Kelly, R. C.; Mitchell, M. A.; Wicnienski, N. A.; Gebhard, I.; Johnson, P. D.; Bradford, V. S. *J. Am. Chem. Soc.* **1990**, *112*, 4633.
(14) Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 4623.
(15) Boger, D. L.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1431. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 3980.
(16) Boger, D. L.; Munk, S. A. *J. Am. Chem. Soc.* **1992**, *114*, 5487. Boger, D. L.; Munk, S. A.; Ishizaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 2779.
(17) Boger, D. L.; Palanki, M. S. S. *J. Am. Chem. Soc.* **1992**, *114*, 9318. Boger, D. L.; Johnson, D. S.; Palanki, M. S. S. *Bioorg. Med. Chem.* **1993**, *1*, 27. Boger, D. L. *Pure Appl. Chem.* **1993**, *65*, 1123.
(18) Boger, D. L.; Yun, W.; Johnson, D. S. Unpublished studies. Boger, D. L. *Pure Appl. Chem.*, in press.
(19) Warpehoski, M. A.; Harper, D. E.; Mitchell, M. A.; Monroe, T. J. *Biochemistry* **1992**, *31*, 2502.
(20) Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. *J. Am. Chem. Soc.*, in press.
(21) Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 4796. Review: Coleman, R. S.; Boger, D. L. In *Studies in Natural Products Chemistry*; Rahman, A-ur-, Ed., Elsevier: Amsterdam, 1989; Vol. 3, p 301.
(22) Boger, D. L.; Invergo, B. J.; Coleman, R. S.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C.; Leong, T.; McLaughlin, L. W. *Chem.-Biol. Interact.* **1990**, *73*, 29. Boger, D. L.; Sakya, S. M. *J. Org. Chem.* **1992**, *57*, 1277.
(23) Boger, D. L. In *Heterocycles in Bioorganic Chemistry*; Bergman, J., Van der Plas, H. C., Simonyi, M., Eds.; Royal Society of Chemistry: Cambridge, 1991. Boger, D. L. In *Proc. Robert A. Welch Found. Conf. Chem. Res.* 35th *Chem. Front. Med.* **1991**, *35*, 137.
(24) In these studies, we have also suggested that the extent of the noncovalent binding stabilization may be directly related to the unique delayed toxicity of (+)-CC-1065 and (+)-CPI-$CDPI_2$ which is not embodied in simpler analogs of (+)-CC-1065 or duocarmycin A and SA. As we have suggested elsewhere,[14,23] the delayed toxicity of CC-1065 may be a consequence of the extent of the noncovalent binding stabilization which renders the DNA alkylation reaction essentially irreversible.