

## Degradation of DNA–RNA Hybrids by Bleomycin: Evidence for DNA Strand Specificity and for Possible Structural Modulation of Chemical Mechanism

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Received July 13, 1987

In 1975 Haidle and Bearden<sup>1</sup> reported that bleomycin, a known cleaver of double-stranded DNA,<sup>2</sup> specifically degraded the poly(dT) strand of the hybrid poly(rA)·poly(dT). This conclusion was based on an analysis of a Cs<sub>2</sub>SO<sub>4</sub> equilibrium density-gradient profile, but no detailed product determinations were performed.<sup>3</sup> No further studies of bleomycin action on hybrids have been reported—a surprising circumstance in light of the intense mechanistic scrutiny this drug has received<sup>4,5</sup> and of the burgeoning appreciation of the structural heterogeneity of various DNA forms.<sup>6</sup> We here report our initial studies on the reaction of bleomycin, Fe(II), and O<sub>2</sub> with poly(dA)·poly(dT), poly(rA)·poly(dT), and poly(dA)·poly(rU). We also offer an interpretation of the results in the light of recent thinking on the bleomycin reaction mechanism and on hybrid structure.

HPLC profiles of a typical series of reactions are shown in Figure 1.<sup>7</sup> Fe(II)·bleomycin-mediated degradation of the al-

Table I. Quantitation of Monomeric Products from Bleomycin Reactions

substrate	amount <sup>a</sup> (nmol)						T + TP/ A + AP
	A	AP	T	TP	A/AP	T/TP	
poly(dA·dT)	1.0	1.8	7.0	6.0	0.6 <sup>b</sup>	1.2	4.6
poly(dA)·poly(dT)	6.0	8.0	5.5	6.0	0.75	0.92	0.82
poly(rA)·poly(dT)			6.5	5.0		1.3	
poly(dA)·poly(rU)	7.0	2.4			2.9		

<sup>a</sup> On the basis of 30 μL of reaction mixture. <sup>b</sup> Probably an underestimate due to errors in the quantitation of small amounts of adenine and adenine propenal for poly(dA·dT).

ternating copolymer poly(dA·dT) under normal atmosphere (Figure 1A) afforded four monomeric products—thymine, adenine, thymine propenal, and adenine propenal, corroborating our previous studies.<sup>5a-d</sup> An identical reaction with poly(dA)·poly(dT) (Figure 1B) afforded, as expected, the same four products. Importantly, while the ratio of thymidine-derived products to adenosine-derived products differed significantly for these two DNAs (an indication of sequence specificity of bleomycin), the internal product partitioning (free base to base propenal) remained fairly constant (Table I). This is consistent with our proposed mechanism of 4'-hydrogen abstraction by activated bleomycin and O<sub>2</sub>-dependent partitioning of the resulting radical species (or equivalent).<sup>5</sup>

Degradation of the hybrid poly(rA)·poly(dT) by activated bleomycin yielded exclusively thymine and thymine propenal with no detectable adenosine-derived products (Figure 1C).<sup>8</sup> In addition, the ratio of thymine to thymine propenal was essentially identical with that found for the two DNAs above (Table I). In order to rule out the possibility of an exaggerated base specificity, the degradation of "inverse" hybrid poly(dA)·poly(rU) was also examined (Figure 1D).<sup>9</sup> Adenine and adenine propenal were the only major monomeric products observed.<sup>10</sup> Of particular significance was the dramatic increase in the amount of adenine produced relative to adenine propenal compared to the DNA models (Table I).<sup>11</sup>

Our findings show conclusively that base and base propenal are formed upon reaction of a hybrid with Fe(II)·bleomycin and O<sub>2</sub> and that DNA strand specificity appears to be essentially complete. It is not clear at present whether this specificity reflects a selectivity in drug binding or an inhibition of hydrogen abstraction from the RNA strand.

The most intriguing observation is the anomalously large amount of adenine, relative to adenine propenal, generated from poly(dA)·poly(rU). This is not easily explicable by our hypothesis of O<sub>2</sub>-dependent 4'-radical partitioning. However, it does suggest a potentially significant modulation of mechanism by nucleic acid structure. X-ray fiber diffraction analysis suggests that the structure of poly(dA)·poly(rU) is polymorphic with a minor groove which is more open than in a B-form though less than in an A-form.<sup>12</sup> Such an opening of the minor groove could lead to an enhanced accessibility of the 1'-hydrogen as well as the 4'-hydrogen to activated bleomycin. While 4'-hydrogen abstraction by activated bleomycin leads to both base and base propenal, abstraction of H-1' would most likely lead to the exclusive release

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(7) A typical reaction mixture (0.1 mL, final vol.) contained 2 mM polymer (nucleotide concentration), 0.6 mM bleomycin (Blenoxane; Bristol Laboratories), 1.2 mM ferrous sulfate, and 10 mM sodium phosphate (pH 7.5). Four aliquots (5 μL each) of 6 mM FeSO<sub>4</sub> were added over a period of 30 min. After an additional 30 min at room temperature, a 30-μL sample was analyzed by HPLC (Rainin Microsorb Short One (3 μm) C<sub>18</sub> reverse phase; 0–100% methanol; 30 min). Control reactions contained no bleomycin. Product analyses were based on 3–4 experiments. Products were identified by coinjection of authentic compounds and quantitated by isolation and UV absorbance and, in the case of base propenals, by the thiobarbituric acid method.<sup>4b</sup> Experimental conditions (low salt concentrations) were chosen to minimize triplex formation: Steeley, H. T., Jr.; Gray, D. M.; Ratliff, R. L. *Nucleic Acids Res.* **1986**, *14*, 10071–10090.

(8) While one would not predict the generation of base propenal from a ribonucleotide moiety, the expectation of free base release should be apparent (see Scheme I).

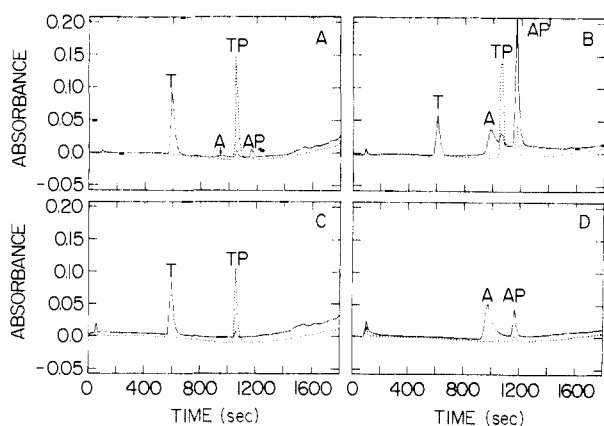
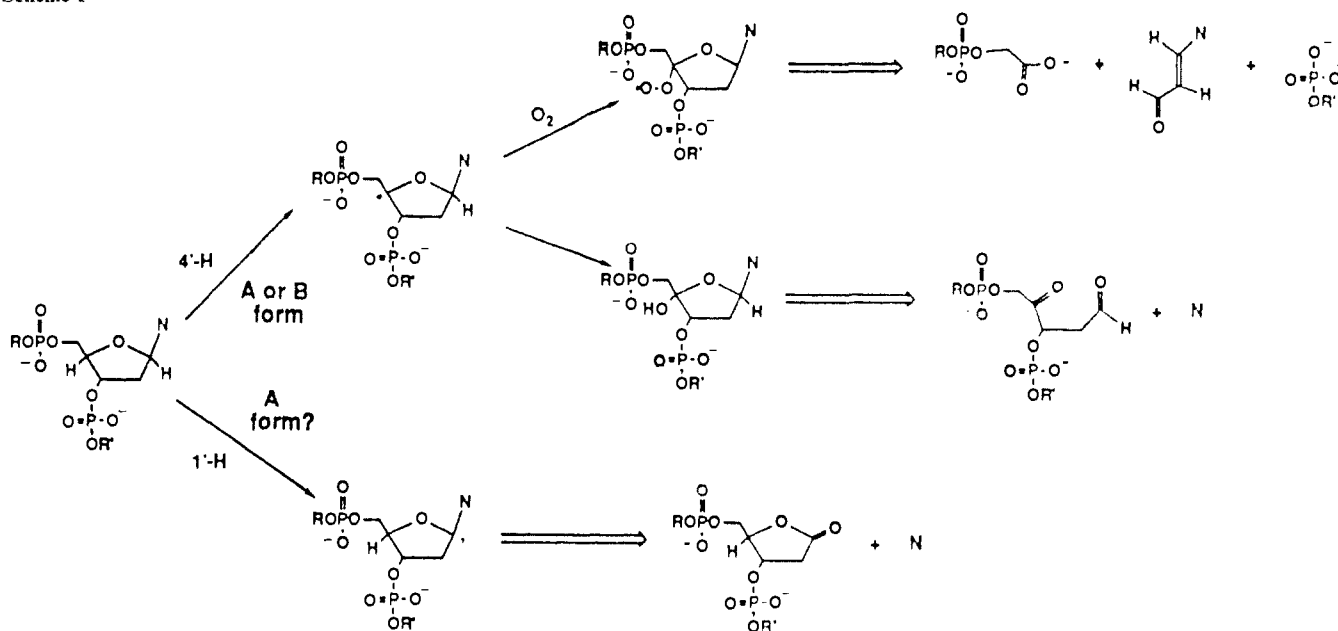
(9) Bleomycin-mediated degradation of uracil-containing DNAs is generally indistinguishable from the corresponding thymine-containing polymer.<sup>1,5a-d</sup>

(10) The retention times for uracil and uracil propenal are 200 and 700 s, respectively.

(11) Under normal atmosphere the ratio of adenine to adenine propenal is usually 1:2 to 1:1 for most DNAs with variability due to inaccuracies in the quantitation of small amounts of products. The 3:1 ratio for poly(dA)·poly(rU) is remarkable.

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Scheme I



**Figure 1.** Reverse phase HPLC profiles for reactions of Fe(II)-bleomycin with A, poly(dA-dT); B, poly(dA)·poly(dT); C, poly(rA)·poly(dT); and D, poly(dA)·poly(rU). Solid lines and broken lines represent the UV absorbance at 260 and 300 nm, respectively: A = adenine; AP = adenine propenal; T = thymine; TP = thymine propenal.

of free base (Scheme I). Thus, the product ratio of poly(dA)·poly(rU) could be explained by an initial partitioning between 4'-hydrogen and 1'-hydrogen abstraction.<sup>13</sup> It is particularly significant that the product ratio for poly(rA)·poly(dT) is similar to those found for the B-form DNAs. This hybrid is believed to be a B-form.<sup>6a,14</sup>

While this hypothesis is speculative, it does suggest that bleomycin may serve as a useful probe of nucleic acid structure and may explain the observation by others of an apparent  $O_2$ -independent release of free base by using heterogeneous DNA.<sup>4e</sup> Our recent preliminary studies indicate that a significant portion of adenine release from poly(dA)·poly(rU) is insensitive to high  $O_2$  concentrations as compared to the other duplexes, an observation consistent with 1'-hydrogen abstraction. Application of

the isotopic analyses developed by us to these hybrids should be informative and are in progress.

**Acknowledgment.** This work was supported by a grant from the National Institutes of Health (GM 34454).

### Electrochemistry at the Water/Air Interface. Lateral Electron Transport in Langmuir Monolayers

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Received November 6, 1987

Revised Manuscript Received January 15, 1988

In our recent reports, we have described electrochemical measurements of the lateral charge transport in self-assembled bilayers of electrochemically active amphiphiles.<sup>1-3</sup> In those measurements, a microporous aluminum oxide template was used to assemble bilayers composed of octadecyltrichlorosilane and an octadecyl derivative of naphthoquinone, ferrocene, or methylviologen. In all three systems, the lateral charge transport is sustained by the translational diffusion of the electroactive amphiphiles along the bilayer. In this communication, we report the results of the electrochemical measurements of lateral electron transport in amphiphilic monolayers spread at the water/air interface under controlled surface pressure conditions. In these experiments, a microelectrode is oriented *perpendicular* to the water/air interface and addresses an electroactive surfactant monolayer at that interface. In a system involving ferrocenylmethylidimethyloctadecylammonium perchlorate ( $C_{18}Fc-ClO_4$ ) monolayer spread at the water/air interface, the electron transport occurs via lateral electron hopping in the monolayer.

The crucial part of the experiment involves the construction of a microelectrode and its positioning at the water/air interface. The microelectrodes are prepared by vapor-deposition of a 300 Å gold film at an ca.  $1 \times 2 \text{ cm}^2$  glass slide. The surface of the gold film is then insulated and rendered hydrophobic by spin-coating an approximately 2000 Å thick layer of polystyrene. By

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