Nucleic Acids Research, 1996, Vol. 24, No. 17 **3399–3406**

Fe·bleomycin as a probe of RNA conformation

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Received May 9, 1996; Revised and Accepted July 11, 1996

ABSTRACT

Two crystallographically defined tRNAs, yeast $tRNA^{Asp}$ and $tRNA^{Phe}$, were used as substrates for oxidative cleavage by Fe·bleomycin to facilitate definition at high resolution of the structural elements in RNAs conducive to bleomycin binding and cleavage. Yeast $tRNA^{Asp}$ underwent cleavage at G_{45} and U_{66}; yeast $tRNA^{Phe}$ was cleaved at four sites, namely G_{19}, A_{31}, U_{52} and A_{66}. Only two of these six sites involved oxidative cleavage of a 5′-G·Pyr-3′ sequence, but three sites were at the junction between single- and double-stranded regions of the RNA, consistent with a binding model in which the bithiazole + C-terminal substituent of bleomycin bind to minor groove structures on the RNA. Also studied were four tRNA transcripts believed on the basis of biochemical and chemical mapping experiments to share structural elements in common with the mature tRNAs. Cleavage of these tRNAs by Fe·bleomycin gave patterns of cleavage very different from each other and than those of the mature tRNAs. This observation suggests strongly that Fe·bleomycin cannot be used for chemical mapping in the same fashion as more classical reagents, such as Pb^{2+} or dimethyl sulfate. However, the great sensitivity of Fe·bleomycin to changes in nucleic acid structure argues that those species which do show similar patterns of cleavage must be very close in structure.

INTRODUCTION

The bleomycin (BLM) group of antitumor antibiotics (1,2; Fig. 1) are believed to mediate their therapeutic effects at the level of nucleic acid modification (3–8). In the presence of a suitable redox-active metal ion and oxygen, bleomycin mediates DNA strand scission in a sequence-selective fashion; 5′-GC-3′ and 5′-GT-3′ sequences are preferred sites of cleavage (3–6).

BLM also causes RNA strand scission, utilizing the same cofactors required for DNA degradation. A survey of potential RNA substrates has documented the ability of Fe(II)·BLM + O_2 to effect the strand scission of tRNA (7,9–11), tRNA precursor transcript (7,9–14), mRNA (12,15), rRNA (11) and RNA–DNA heteroduplex structures (16), as well as the RNA nucleotide in a chimeric octanucleotide (12,17).

Although the available evidence suggests that Fe·BLM binds to RNA more avidly than it binds to DNA of similar structure (18), the cleavage of RNA by Fe·BLM is much more selective than the cleavage of DNA (1,10–14,16,18). This greater selectivity is evident both from the existence of RNAs that are not substrates for Fe·BLM and in the relatively small number of sites at which cleavage occurs in those RNAs that are substrates (7,10–16,18). Further, while 5′-GC-3′ and 5′-GU-3′ sites are among those cleaved with greater frequency in RNA by Fe·BLM, there are numerous examples of RNA cleavage at sites that must involve recognition of shape, rather than sequence (7,11).

In order to better define the structural parameters in RNA conducive to cleavage by Fe·BLM, we have studied the effect of Fe·BLM on yeast $tRNA^{Asp}$ and $tRNA^{Phe}$, two tRNAs whose structures have been defined at high resolution by X-ray crystallography (19–23) and whose solution structures have been studied (24–30). Also employed as substrates for Fe·BLM were representative examples of a set of $tRNA^{Asp}$ constructs lacking post-transcriptional modifications, some of which also contain structural elements characteristic of yeast $tRNA^{Phe}$ (31–35). These species undergo facile aminoacylation by the cognate aminoacyl-tRNA synthetases although they exhibit significant conformational alterations, as revealed by direct probing of tRNA transcript structures (32,34). By the use of this set of tRNA transcripts, whose structures represent part of a structural continuum from that of yeast $tRNA^{Asp}$ to yeast $tRNA^{Phe}$, the effect of systematic alteration of structure on susceptibility to cleavage by Fe·BLM could be assessed.

Presently, we describe the sites of Fe·BLM-mediated cleavage of yeast $tRNA^{Asp}$ and $tRNA^{Phe}$, as well as four structurally related tRNA transcripts. A number of sites of cleavage of the mature tRNAs are fully consistent with a recently reported model for DNA binding by BLM (36,37). Cleavage of the unmodified transcripts reflected a substantial sensitivity of Fe·BLM to alteration of substrate structure, but also some commonality among the observed sites of cleavage.

MATERIALS AND METHODS

Blenoxane was obtained from Bristol Laboratories and was fractionated as described (38,39) to afford bleomycin A_2. Calf

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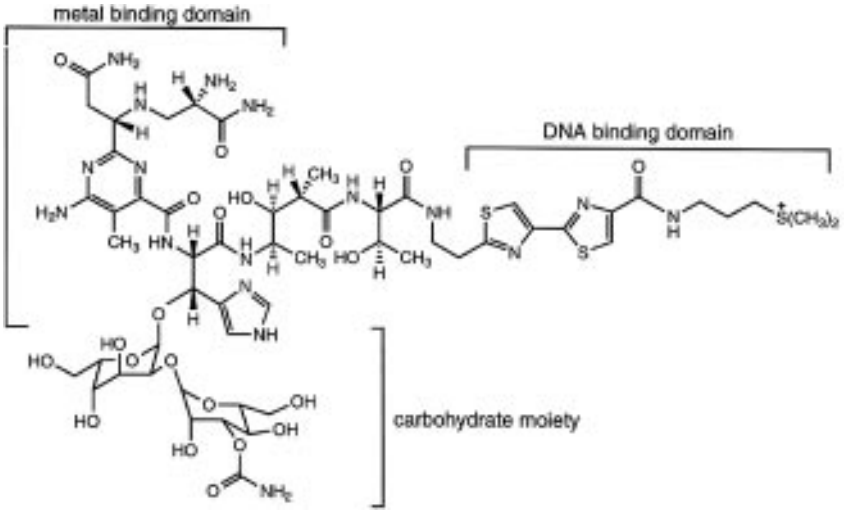

Figure 1. Structure of bleomycin A_2. The functional domains are indicated.

intestinal alkaline phosphatase, T7 RNA polymerase, restriction endonuclease *Bst*NI and *Saccharomyces cerevisiae* $tRNA^{Phe}$ were purchased from Boehringer Mannheim. T4 polynucleotide kinase, T4 RNA ligase and the RNA sequencing system were obtained from Bethesda Research Laboratories; the RNase inhibitor (RNasin) was from Promega. Qiagen columns used for the purification of RNA transcripts were purchased from Qiagen Inc. [γ-^{32}P]ATP (>7000 Ci/mmol) was from ICN Radiochemicals. $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (Alfa Products) was used to prepare aqueous solutions for admixture with bleomycin; these were made immediately prior to use.

Preparation of nucleic acids

Yeast $tRNA^{Asp}$ was isolated from unfractionated brewer's yeast tRNA as described (27). Plasmids containing genes encoding wild-type and modified $tRNA^{Asp}$ constructs were prepared as described (32,33). Plasmid DNA was isolated from *Escherichia coli* by the method of Sambrook *et al.* (40) using Qiagen columns for final purification.

Plasmid DNAs (30–50 µg) were linearized using 5 U *Bst*NI in 50 mM Tris–HCl, pH 7.5, containing 1 mM dithiothreitol, 10 mM $MgCl_2$ and 100 mM NaCl at 60°C for 2 h. The $tRNA^{Asp}$ transcripts were prepared by *in vitro* RNA transcription in the presence of T7 RNA polymerase (32); they were purified using Qiagen columns (11).

Radiolabeling of tRNAs

The $tRNA^{Asp}$ transcripts were dephosphorylated by incubation in 20 µl (total volume) 50 mM Tris–HCl, pH 9.0, containing 3 U calf intestinal alkaline phosphatase, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 1 mM spermidine. The incubation was carried out at 37°C for 1 h and the dephosphorylated $tRNA^{Asp}$ transcript was 5′-^{32}P-end-labeled via the agency of 20–30 U T4 polynucleotide kinase in 50 mM Tris–HCl, pH 7.6, containing 10 mM $MgCl_2$ and 0.3 mCi [γ-^{32}P]ATP at 37°C for 2–3 h.

Mature yeast $tRNA^{Asp}$ and $tRNA^{Phe}$ were radiolabeled at the 3′-end by the use of 5′-[^{32}P]pCp. The latter was prepared in a reaction mixture (15 µl total volume) containing 40 mM Tris–HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 6.7 mM dithiothreitol, 1.5 mM 3′-CMP, ~0.5 mCi [γ-^{32}P]ATP and 20 U T4 polynucleotide kinase. The solution was incubated at 37°C for 2–3 h and then at 75°C for 20 min to inactivate the polynucleotide kinase. The tRNA substrate was 3′-^{32}P-end-labeled in a reaction mixture (50 µl total volume) containing the 5′-[^{32}P]pCp prepared above, 10 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM ATP, 0.4 mg/ml bovine serum albumin, 30 U T4 RNA ligase and ~3–6 µg $tRNA^{Asp}$ or $tRNA^{Phe}$ in 50 mM HEPES buffer, pH 8.3. The reaction mixture was incubated at 4°C for 18 h and the radiolabeled tRNA was purified by polyacrylamide gel electrophoresis.

Fe(II)·bleomycin-mediated tRNA cleavage

Reaction mixtures (5 µl total volume) contained ~0.5–5 µM labeled tRNA (final nucleotide concentration) in 5 mM Na phosphate, pH 7.5. The reactions were initiated by the simultaneous addition of equimolar BLM A_2 and Fe^{2+} (as a freshly prepared solution) to the final concentrations indicated in the figure legends. Reactions were incubated at 22°C for 15 min, stopped by the addition of 1 mM EDTA and then analyzed by electrophoresis on 10–20% denaturing polyacrylamide gels.

RESULTS

Strand scission of mature tRNAs by Fe(II)·BLM A_2

The cleavage of yeast cytoplasmic $tRNA^{Asp}$ by Fe(II)·BLM A_2 was studied using a mature tRNA radiolabeled at its 3′-terminus by incubation with 5′-[^{32}P]pCp in the presence of T4 RNA ligase. As shown in Figure 2, this substrate was cleaved at two sites shown by comparison with RNA sequencing lanes to be G_{45} and U_{66}. The cleavage of yeast $tRNA^{Phe}$ was studied in the same fashion (Fig. 3). Four sites of cleavage were observed for $tRNA^{Phe}$; RNA sequence analysis indicated that these involved G_{19}, A_{31}, U_{52} and A_{66}. Because an earlier report (10) had indicated that Fe(II)·BLM, apparently employed as an unfractionated mixture of congeners, effected cleavage of $tRNA^{Phe}$ only at A_{31} and G_{53}, we studied the cleavage of this substrate over a 50–500 µM range of Fe(II)·BLM A_2 concentrations and verified

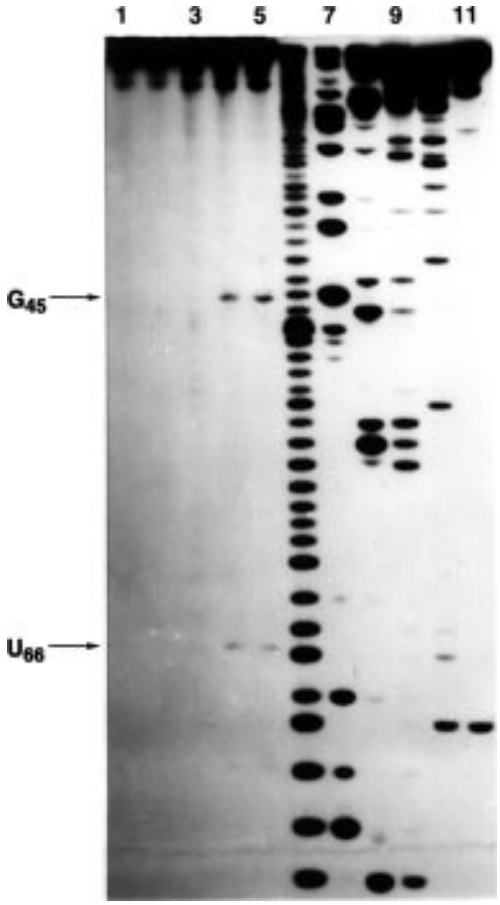

Figure 2. Cleavage of mature yeast tRNA^{Asp} by Fe(II)·BLM A_2. Samples of 3′-^{32}P-end-labeled tRNA^{Asp} were treated with Fe(II)·BLM A_2 as described under Materials and Methods. Lane 1, tRNA alone (~1.0 μM final nucleotide concentration); lane 2, 250 μM BLM A_2; lane 3, 250 μM Fe^{2+}; lane 4, 500 μM Fe(II)·BLM A_2; lane 5, 250 μM Fe(II)·BLM A_2; lane 6, alkaline hydrolysis; lanes 7–11, G, A>G, U+A, C+U and C sequencing lanes respectively. The sites of Fe(II)·BLM A_2 cleavage are denoted by arrows.

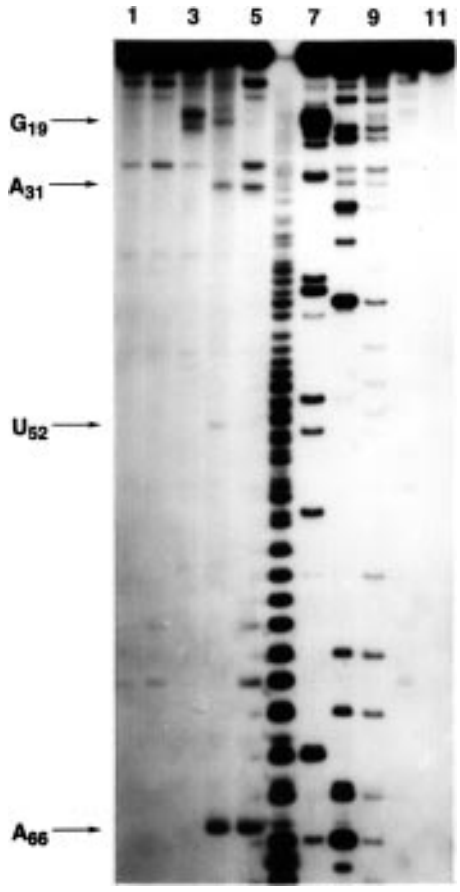

Figure 3. Cleavage of mature yeast tRNA^{Phe} by Fe(II)·BLM A_2. Samples of 3′-^{32}P-end-labeled tRNA^{Phe} were treated with Fe(II)·BLM A_2 as described under Materials and Methods. Lane 1, tRNA alone (2.5 μM final nucleotide concentration); lane 2, 250 μM BLM A_2; lane 3, 250 μM Fe^{2+}; lane 4, 500 μM Fe(II)·BLM A_2; lane 5, 250 μM Fe(II)·BLM A_2; lane 6, alkaline hydrolysis; lanes 7–11, G, A>G, U+A, C+U and C sequencing lanes respectively. The four sites of Fe(II)·BLM A_2-mediated cleavage are denoted with arrows.

the sites of cleavage in replicate experiments. The assignment of sites of cleavage took cognizance of the fact that oxidative cleavage of RNA by Fe(II)·BLM produces fragments containing 5′-phosphate termini, while the base-specific sequencing reactions afford products containing 5′-hydroxyl termini (7,11,12,17).

The sites of cleavage of these two tRNAs are summarized in Figure 4. For tRNA^{Asp}, one of the sites of cleavage involved a 5′-GU-3′ sequence at the junction between single- and double-stranded regions of the tRNA (7,11,12). For yeast tRNA^{Phe}, only one of the four sites cleaved involved a 5′-GU-3′ sequence, but another two of the four were located at the putative junctions between single- and double-stranded regions of the tRNA. The most obvious similarity between the two species was the cleavage observed at position 66; this involved a 5′-GU-3′ sequence in tRNA^{Asp} and a 5′-GA-3′ sequence in tRNA^{Phe}.

Cleavage of *in vitro* tRNA transcripts

Also utilized as substrates for cleavage by Fe(II)·BLM were four *in vitro* tRNA^{Asp} transcripts whose primary sequences were varied systematically to identify the structural elements essential for recognition by aspartyl-tRNA synthetase (32,34) (Fig. 5).

Transcript **A** is closest in structure to wild-type tRNA^{Asp}. Although not post-transcriptionally modified, it has the same sequence as yeast tRNA^{Asp} with the exception that the canonical U_1-A_{72} base pair has been replaced by G_1-C_{72} to facilitate *in vitro* transcription (32). In previous studies it has been shown that the tRNA^{Asp} transcript is aminoacylated efficiently by yeast aspartyl-tRNA synthetase and that the G_1-C_{72} substitution for the wild-type U-A base pair had no significant effect on tRNA transcript aminoacylation (41).

As shown in Figure 6, 5′-^{32}P-end-labeled tRNA^{Asp} transcript **A** underwent cleavage at four sites by Fe(II)·BLM A_2. RNA sequence analysis indicated that cleavage had occurred at U_{16}, C_{29}, C_{38} and A_{46}; the positions of cleavage were found to be the same at 50–500 μM Fe(II)·BLM A_2 concentrations (data not shown). While cleavage occurred within putative single- and double-stranded regions of the tRNA, none of the sites of cleavage was in proximity to U_{66}, a site of cleavage of the mature tRNA^{Asp}. However, transcript **A** was cleaved at A_{46}, which is close to the site of cleavage of mature tRNA^{Asp} at G_{45}. The

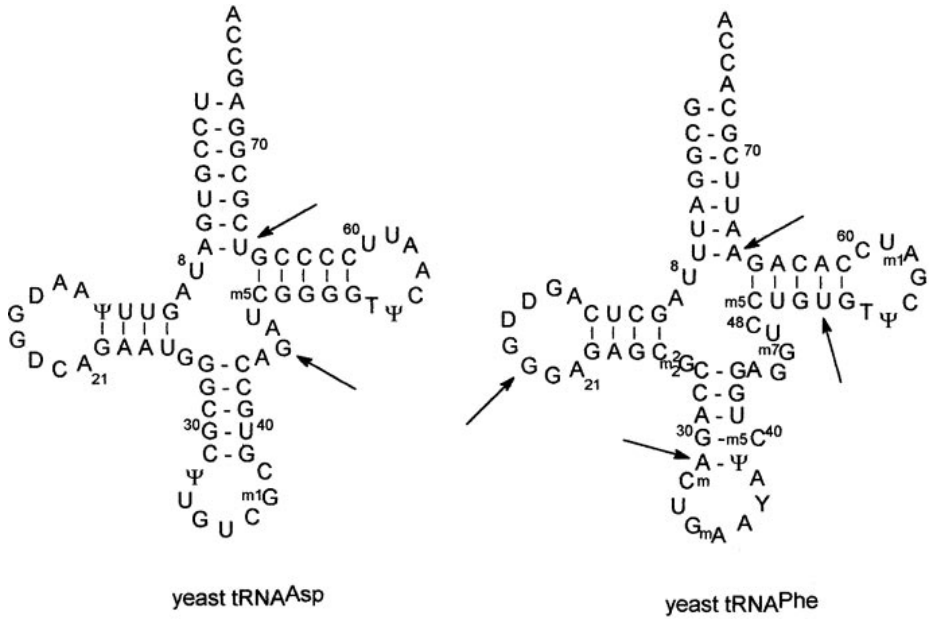

Figure 4. Structures of yeast $tRNA^{Asp}$ (left) and yeast $tRNA^{Phe}$ (right). The sites of Fe(II)·BLM A_2-mediated cleavage are indicated by arrows.

greater number of Fe·BLM cleavage sites observed for the $tRNA^{Asp}$ transcript as compared with mature $tRNA^{Asp}$ is superficially consistent with the results of chemical mapping experiments that reflect a more 'relaxed' structure for the transcript, which consequently undergoes reaction at more sites. However, the reagents employed for chemical mapping, which included Pb^{2+}, dimethyl sulfate and diethyl pyrocarbonate, gave cleavage patterns that reflected more similarities than differences between the mature and unmodified $tRNA^{Asp}$ (32).

A second unmodified $tRNA^{Asp}$ used as a substrate for Fe(II)·BLM A_2 was transcript **F**. Beyond the changes noted above for transcript **A**, this transcript contains two structural changes characteristic of yeast $tRNA^{Phe}$, namely an altered D loop and a fifth nucleotide in the variable loop (34). As shown in Figure 7, cleavage of this transcript obtained at five sites, namely U_{17}, G_{19}, A_{21} and U_{25}, all of which are located in the dihydrouridine stem and loops and G_{50}. The last of these, involving cleavage of a guanosine within the stem of the TΨC stem–loop structure, was the strongest of the cleavage sites in replicate experiments. It is interesting that one of the sites of cleavage in the dihydrouridine loop, G_{19}, was also cleaved in mature yeast $tRNA^{Phe}$. The strong cleavage at G_{50} in transcript **F** was also close to the observed U_{52} cleavage site in mature $tRNA^{Phe}$.

Two additional $tRNA^{Asp}$ transcripts were also studied as substrates for cleavage by Fe(II)·BLM A_2 (Fig. 5). These are transcripts **B** and **D**, each of which contains only one of the two alterations by which transcripts **A** and **F** differ. Thus, transcript **B** contains a fifth nucleotide in the variable loop (identical with the sequence in **F**) and transcript **D** has an altered dihydrouridine loop with the same sequence as transcript **F**. When treated with Fe(II)·BLM A_2 under the same conditions as the other tRNAs, transcript **B** was cleaved at eight sites while transcript **D** was cleaved only at one site (Fig. 5).

For transcript **B**, the sites of cleavage included U_{16}, C_{29}, C_{31}, C_{38}, C_{42}, U_{54}, U_{66} and C_{69}. Thus a single nucleotide addition in the variable loop rendered this transcript more susceptible to cleavage by Fe·BLM than transcript **A**. Nonetheless, both transcripts were cleaved at U_{16}, C_{29} and C_{38}, reflecting the great similarity in these structures. The only site cleaved in transcript **A** that was not cleaved in **B** involved A_{46}, which is immediately adjacent to the site of modification in transcript **B**. Two sites of cleavage were also found for transcript **B** within the acceptor stem; one of these was identical to the site of cleavage of mature $tRNA^{Asp}$.

Transcript **D**, which has a dihydrouridine loop identical in structure to transcript **F**, failed to undergo cleavage at all within this part of the molecule, even though transcript **F** was cleaved at four sites within the dihydrouridine stem–loop structure. The single site of cleavage of transcript **D** involved A_{46}, which was also cleaved in transcript **A**. The single site of cleavage of this transcript in comparison with the other transcripts of quite similar primary sequence underscores the fact that the 'relaxed' structures established for tRNA transcripts as compared with the mature species (32,41) do not constitute the basis for tRNA recognition and cleavage by Fe(II)·BLM A_2.

DISCUSSION

The recognition of tRNAs (M_r ~25 000) by the polypeptide-derived bleomycin antibiotics (M_r ~1500) represents an interesting example of specific polypeptide–nucleic acid interaction. The modest sizes of both species, relative to the proteins and nucleic acids typically involved in specific recognition events, makes this an attractive system for the definition of molecular strategies for nucleic acid recognition.

Previous studies of the cleavage of tRNAs and tRNA precursor transcripts by Fe(II)·BLM A_2 have been characterized by remarkable selectivity; there are few sites of cleavage and many tRNAs are not substrates at all (10–14). The sites of cleavage often involve 5′-GC-3′ and 5′-GU-3′ sequences or occur at the junction between single- and double-strand regions of the tRNAs. However, there are numerous exceptions and no apparent pattern in the choice of sites of cleavage in any given tRNA. In the present

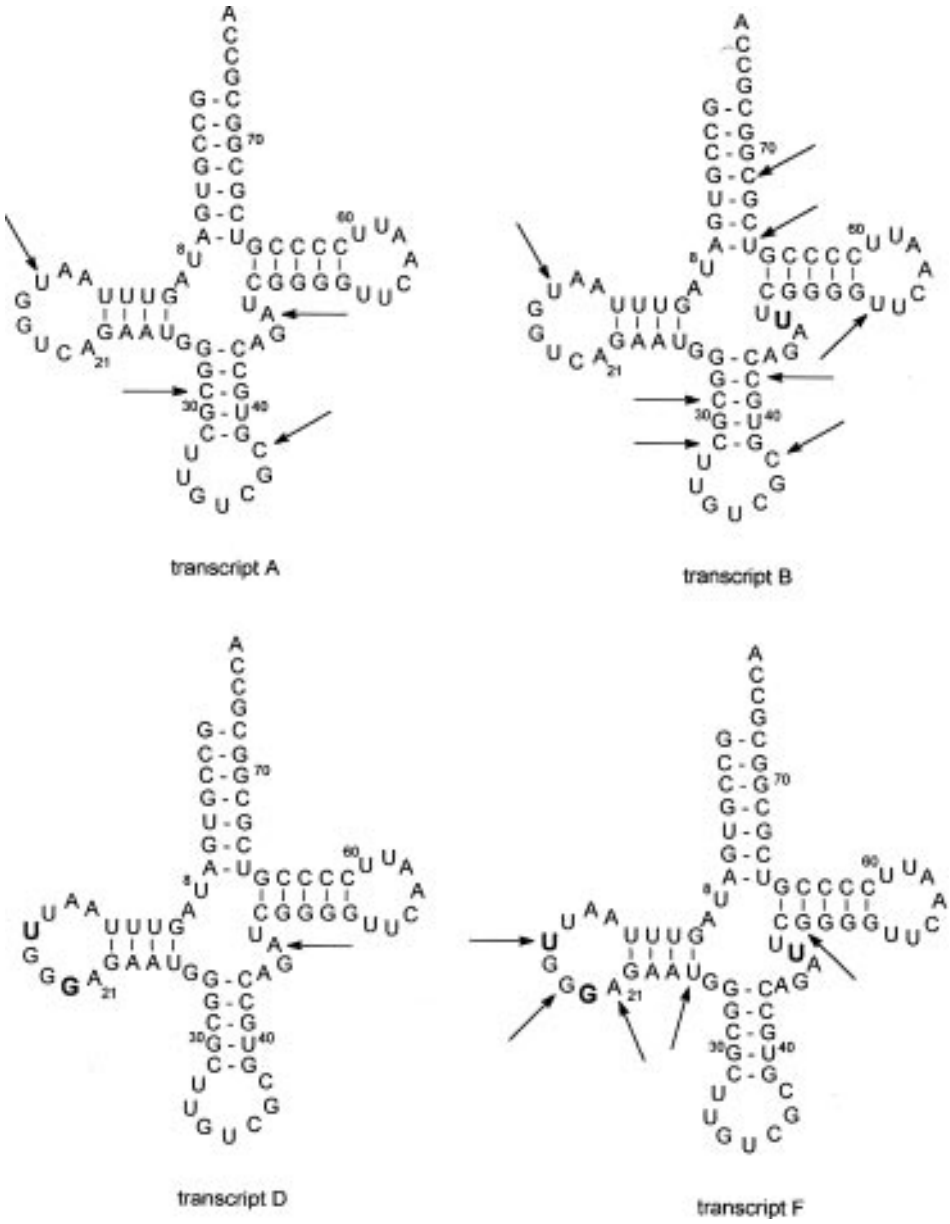

Figure 5. Structures of four tRNA transcripts illustrating (arrows) the sites of cleavage by Fe(II)·BLM A_2. Nomenclature for the transcripts was based on Gieg *et al.* (31). Transcript **A** has the same sequence as mature yeast $tRNA^{Asp}$, but containing a G_1-C_{72} base pair to facilitate transcription in lieu of the canonical U_1-A_{72} base pair. Relative to transcript **A**, transcript **B** contains an additional nucleotide (U_{47}) in the variable loop, while transcript **D** has an altered dihydrouridine loop. Transcript **F** contains both the additional nucleotide in the variable loop and an altered dihydrouridine loop.

study we have employed two mature tRNAs whose structures have been determined by X-ray crystallography and four tRNA transcripts of related structure in an effort to define the recognition elements employed by Fe(II)·BLM A_2 in choosing sites for cleavage in RNA substrates.

The three-dimensional, crystallographically determined structures of yeast $tRNA^{Asp}$ (form A; 42–44) and $tRNA^{Phe}$ (19–23) are shown in Figure 8; the sugar moieties that undergo oxidative cleavage by Fe(II)·BLM A_2 are indicated in white. Both tRNA structures show the characteristic L-shaped folding, with the TΨC and acceptor stems forming a helix that constitutes the upper arm of the L; the dihydrouridine and anticodon stems form the descending arm. The anticodon loop and 3′-CCA terminus of the acceptor stem define the ends of the L structure, with the TΨC and dihydrouridine loops interacting to form the hinge region of the tRNA molecules.

The A_{31} cleavage site in $tRNA^{Phe}$ is located in the anticodon stem in the descending arm of the L structure, immediately adjacent to the anticodon loop. The remaining three sites are located in the upper arm of the tRNA structure. The sites of cleavage within U_{52} and A_{66} are located in the minor groove formed by the helices of the TΨC and acceptor stems. Interestingly, the A_{31}, U_{52} and A_{66} cleavage sites were each preceded by a guanosine within the helical domain. While the G_{19} residue is unique in that it is not located immediately adjacent to a helix, this guanosine is located in a highly negatively charged region of the molecule, as indicated by calculations of electrostatic potential (45).

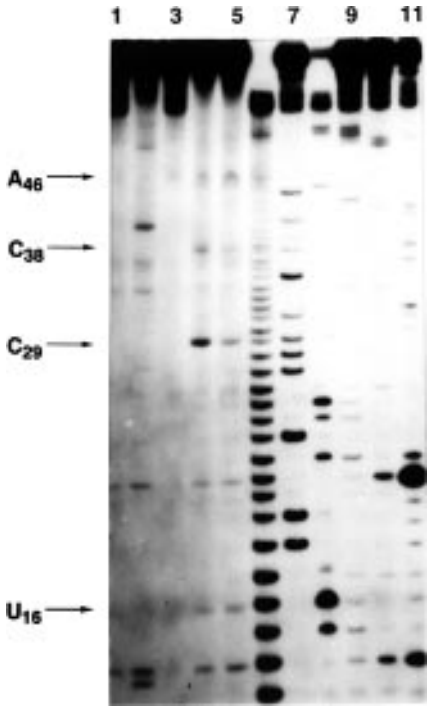

Figure 6. Cleavage of tRNA^{Asp} transcript **A** by Fe(II)·BLM A_2. Samples of 5′-^{32}P-end-labeled tRNA transcript **A** were treated with Fe(II)·BLM A_2 as described. Lane 1, tRNA transcript alone (~2 µM final nucleotide concentration); lane 2, 250 µM BLM A_2; lane 3, 250 µM Fe^{2+}; lane 4, 500 µM Fe(II)·BLM A_2; lane 5, 250 µM Fe(II)·BLM A_2; lane 6, alkaline hydrolysis; lanes 7–11, G, A>G, U+A, C+U and C sequencing lanes respectively. The four sites of Fe(II)·BLM A_2-mediated cleavage are denoted by arrows.

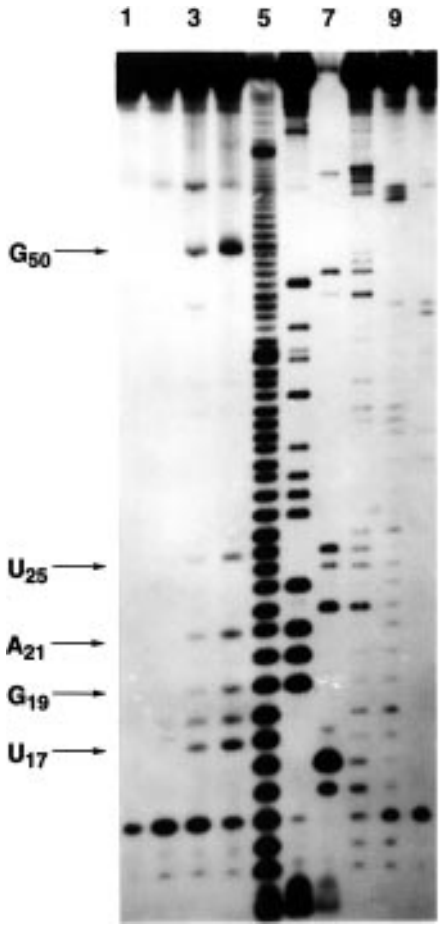

Figure 7. Cleavage of tRNA^{Asp} transcript **F** by Fe(II)·BLM A_2. Samples of 5′-^{32}P-end-labeled tRNA transcript **F** were treated with Fe(II)·BLM A_2 as described under Materials and Methods. Lane 1, tRNA transcript alone (~1 µM final nucleotide concentration); lane 2, 250 µM BLM A_2; lane 3, 500 µM Fe(II)·BLM A_2; lane 4, 250 µM Fe(II)·BLM A_2; lane 5, alkaline hydrolysis; lanes 6–10, G, A>G, U+A, C+U and C sequencing lanes respectively. The five sites of cleavage are denoted by arrows.

One site of cleavage of tRNA^{Asp} was found to be at U_{66} (Figs 2 and 4). As is clear from Figure 8, the sugar moiety of this nucleoside is also located in the minor groove of a helix formed by the acceptor stem of the tRNA. This site of cleavage is in the same position as a site of cleavage (A_{66}) in tRNA^{Phe}.

Also studied was the Fe·BLM-mediated degradation of four tRNA^{Asp} transcripts. Since mature tRNA^{Asp} has only eight post-transcriptionally modified nucleotides, it might have been anticipated that cleavage of the tRNA transcripts would closely parallel cleavage of the mature tRNA. As is clear from a comparison of Figures 4 and 5, this was not the case. None of the transcripts was cleaved at G_{45} and only one was cleaved at U_{66}. It has been noted previously for tRNA^{Asp} (32,41) and tRNA^{Phe} (46,47) that the unmodified transcripts have more ‘relaxed’ or open structures than the fully modified species, a parameter that could account for the differences in sites of cleavage between modified and unmodified tRNAs. In fact, transcript **A**, whose primary sequence is closest to that of mature tRNA^{Asp}, was cleaved at four sites, consistent with the presence of a more open structure. However, transcript **D**, whose structure differs from that of **A** solely within the dihydrouridine loop, was cleaved at only a single site by Fe(II)·BLM A_2.

Comparison of the sites of cleavage of the four tRNA transcripts, whose structures were varied systematically, did illustrate some sites of cleavage that were common to related structures: the lesions at U_{16}, C_{29} and C_{38} in transcripts **A** and **B** represent one good example. Nonetheless, the more general observation was one of great sensitivity of Fe(II)·BLM A_2 to changes in tRNA structure. Repetition of a number of cleavage experiments with Fe(II)·BLM A_5 indicated the same sites of tRNA cleavage, albeit with somewhat greater efficiency, indicating that the present observations are not limited to a single bleomycin congener.

The absence of any simple correlation between RNA structure and sites of Fe·BLM cleavage indicates that BLM cannot be used for chemical mapping in a fashion analogous to reagents such as Pb^{2+}, dimethyl sulfate and diethyl pyrocarbonate. On the other hand, the great sensitivity of Fe(II)·BLM to even small changes in substrate structure may be of substantial utility in providing a criterion for defining close structural similarity between two species. One example is the surprisingly similar patterns of cleavage noted for a *Bacillus subtilis* tRNA^{His} precursor transcript and its corresponding tDNA^{His} (18).

As noted in previous reports (7,11,12) and quite evident in the patterns of cleavage observed for the six substrate RNAs in the present study, many sites of RNA cleavage occur at the putative junctions between single- and double-stranded regions of the

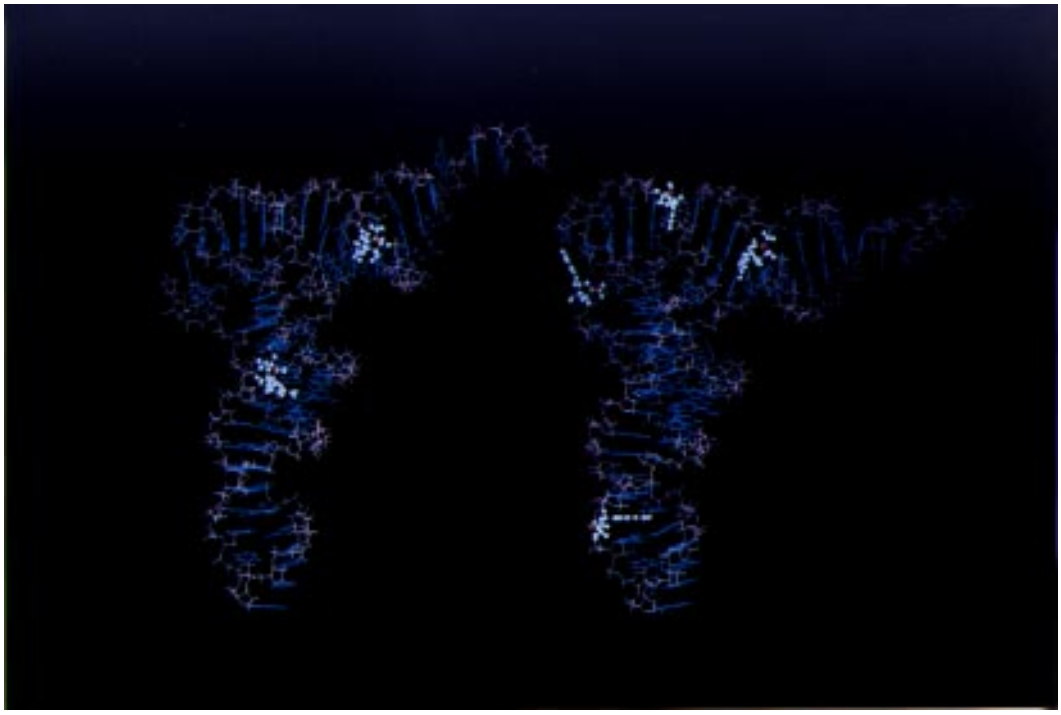

Figure 8. Crystallographically determined structures of yeast $tRNA^{Asp}$ (form A; 42–44) (left) and yeast $tRNA^{Phe}$ (right), showing the major sites of oxidative damage by Fe(II)·BLM A_2. The sugars that undergo Fe·BLM-mediated degradation are shown in white; the C-4′H atoms are shown in red.

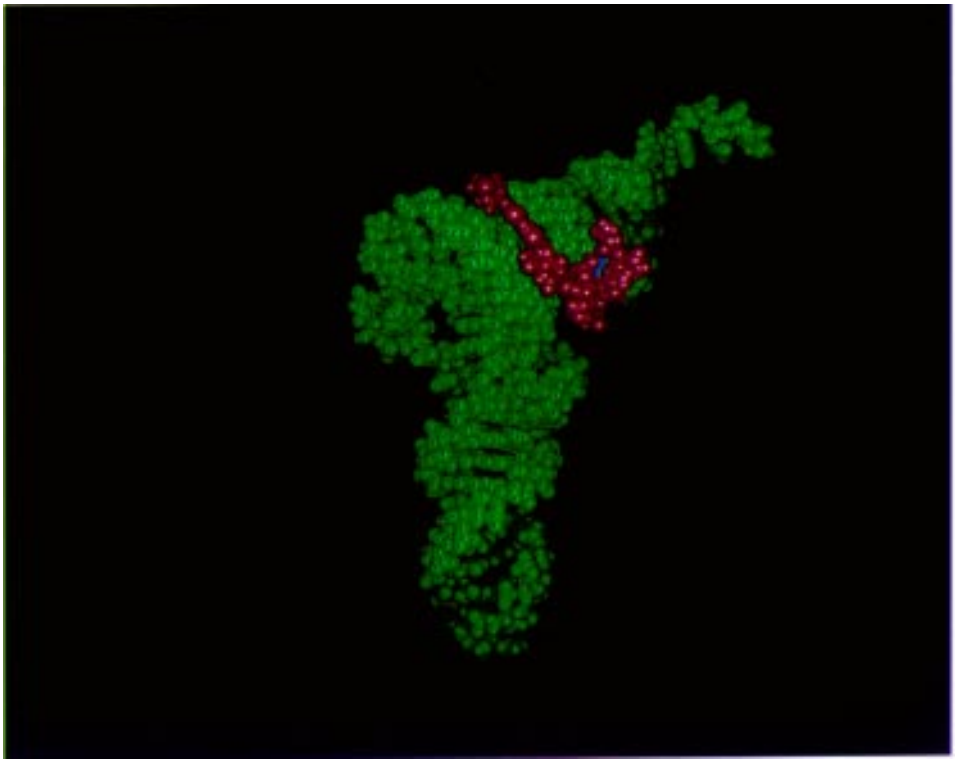

Figure 9. Structure of yeast $tRNA^{Asp}$ showing Fe·BLM A_2 bound in an orientation consistent with the observed degradation of this substrate.

RNAs. As a working hypothesis, we suggest that the interaction of Fe·BLM with such structural elements may reflect binding of the bithiazole + C-terminus of bleomycin to the minor groove of the double-stranded RNA region, in analogy with the binding of Zn·BLM to the minor groove of DNA (36,37). The preference for cleavage at the end of helices could reflect the association of the metal binding domain of Fe·BLM with a structural element wider than a normal minor groove. This would parallel the cleavage of

B-form DNA at 5′-G·Pyr-3′ sequences, which represent the widest portion of the minor groove in DNA, and also at the sites of DNA bulges (48) and at the duplex–triplex junctions for a triple helical DNA structure (49).

On the basis of the foregoing observations, we suggest a model for the Fe(II)·BLM A_2–tRNA interaction; this model is illustrated in Figure 9 for form A of yeast $tRNA^{Asp}$ (42–44). Shown in the figure is a structure for Fe(II)·BLM A_2 based on published metal ligands for Fe(II)CO·BLM (39). This metallobleomycin was docked to the minor groove of $tRNA^{Asp}$ in a fashion that would permit the abstraction of C4′H of U_{66}, as is actually observed experimentally. The Fe·BLM A_2 was docked to the $tRNA^{Asp}$ by steepest descents minimization using distance restraints to guide the minimization within the minor groove. A standard restrained molecular dynamics protocol (50) was then carried out on the docked structure. The resulting structure was then minimized using a conjugate gradient protocol until the maximum derivative was <0.01, affording the structure shown in Figure 9. While not reflecting any direct physical measurement or detailed calculations, this model is consistent with the model developed for Zn(II)·BLM–DNA interaction (36,37) and accommodates the available biochemical data. As such, it may constitute a useful starting point for the design of experiments that can define the nature of BLM–RNA interaction at higher resolution.

ACKNOWLEDGEMENTS

We thank Dr Anne Théobald-Dietrich for purified $tRNA^{Asp}$ and Dr Philippe Dumas for providing us with the crystallographic coordinates of form A of mature yeast $tRNA^{Asp}$. We thank Mr Steven Sucheck and Dr Richard Manderville for assistance with the molecular graphics and Mr Michael Morgan for carrying out some tRNA cleavage experiments. This study was supported at the University of Virginia by research grant CA53913 from the National Cancer Institute and at IBMC, Strasbourg, by a grant from the CNRS.

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