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Site-Specific DNA Photocleavage by Rhodium Intercalators Analyzed by MALDI-TOF Mass Spectrometry

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Oxidative DNA cleavage can be performed using a variety of damaging agents.[1] Our group has focused on rhodium complexes of phenanthrenequinone diimine (phi) that bind to double helical DNA by intercalation. On photoactivation, direct DNA strand cleavage is promoted at the bound site; irradiation at long wavelengths leads also to base oxidation.[2,3] Complexes with sterically more demanding ligands, such as chrysene-5,6-quinone diimine (chrysi), with an expanse exceeding that of the well matched base pair, can intercalate only in mismatched sites, where the base pairs are destabilized; as a result, site specificity is obtained.[4] Recently, we have prepared a novel chrysi complex **1** of Rh containing a pendant peptide for targeting DNA mismatches inside cells.[5] As new derivatives of the DNA-binding metal complexes are prepared, it becomes important to characterize their site specificities. Here we describe a new method to characterize both the site specificity of the DNA cleaving agent and the products.

We describe DNA photocleavage by **1** and other Rh complexes by MALDI-TOF mass spectrometry.[6] Often DNA cleavage is analyzed using gel electrophoresis of ^{32}P-end-labeled DNA.[7] For complexes binding site selectively, however, MALDI-TOF provides a rapid, sensitive method to analyze directly not only site specificities but also cleavage products.

The Rh complex **1** was synthesized by coupling the parent [Rh(phen)(bpy3C)chrysi]$^{3+}$, containing a pendant carboxylate (bpy3C = 4-propionic acid-4′-methyl-2,2′-bpy), and prepared analogously to that described earlier,[8] to the N−(Arg)$_8$Lys−C peptide on the solid phase after activation with HOBT/HBTU. Cleavage from the solid support was performed by standard Fmoc peptide cleavage, and the complex was isolated by HPLC (Supporting Information).

Photocleavage was performed by irradiation after incubation with **1** of an oligomeric DNA duplex (2 μM) containing a single base pair mismatch.[9] After desalting the reaction mixture by the ziptip procedure, MALDI-TOF mass spectra were measured (Figure 2). The uncleaved DNA oligomers (red) are seen at m/z = 5179 (1 DNA A^{1+}), 5166 (2, DNA B^{1+}), 2583 (7, DNA B^{2+}), and 1722 (9, DNA B^{3+}). DNA B^{1+} and DNA B^{2+} have been used for internal mass calibration. Cleavage fragments are also evident at m/z = 3579, 3486, and 3390 (blue) and 1789, 1598 (green), where the former correspond to the A strand cleaved to the 5′-side of the cleavage site and the latter to the A strand cleaved to the 3′-side. These fragments are consistent with cleavage occurring only on DNA strand A, one base pair neighboring the mismatch to the 3′-side. These cleavage products cannot be observed in the absence of light or after irradiation of a reaction mixture in the absence of **1** or **2**. We assign the cleavage product (6 in Figure 2) at m/z = 3390 as a 11-mer DNA with a 3′-phosphate terminus (blue), while the product (10) at m/z = 1598 corresponds to the 5-mer DNA with a 5′-phosphate (green). These are the most common products observed with oxidative DNA damage.[1,10] More surprising are the products at m/z = 3579 (4) and 1789 (8); these correspond in mass to fragments containing 2,3 and 4,5 dehydronucleotide termini

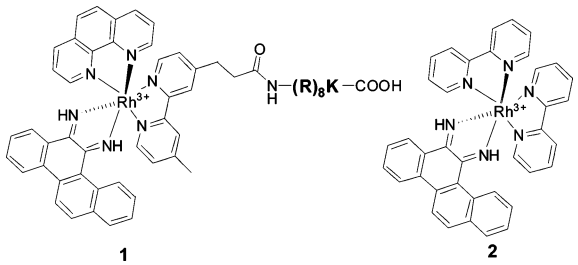

Figure 1. Two bulky metallointercalators targeted to mismatches, **1**, the Rh complex modified with a basic peptide, and **2**, the parent [Rh(bpy)$_2$chrysi]$^{3+}$.

(Supporting Information). A second MALDI-TOF mass measurement after 48 h at ambient temperature shows complete conversion of these products assigned as dehydronucleotides to the phosphate-modified nucleotides (see inset a in Figure 2). We assign peak 5 (m/z = 3486) to a furanone derivative.[1,10] Also evident is a large molecular fragment, 3, that we assign as an oxidation product of DNA A, the 2′-deoxyribonolactone (m/z = 5084).[11]

These cleavage data may be compared to gel electrophoresis analysis of the cleavage reaction. Photocleavage is observed on the same DNA strand (A) at the same position as observed by MALDI-TOF analysis (Figure 3). Interestingly, only a smeared cleavage band is observed when the cleavage reaction is examined by gel electrophoresis directly after the photocleavage, while a sharp cleavage band, which we suggest is the stable 3′-phosphate cleavage product, is observed following a 20 h incubation at 20 °C in the dark after irradiation.[12]

We have also examined DNA photocleavage products using the parent [Rh(bpy)$_2$chrysi]$^{3+}$ by MALDI-TOF analysis. Fragments with masses are shown as inset b in Figure 2. Importantly, equivalent products are observed as with **1**, although here stronger peak intensities for fragments are seen.[13] Although weaker in intensity, analogous intermediates can also be observed for the 5′ cleavage products.[14] Whether these products reflect major or minor groove access depends on the structure of the mismatched site with bound intercalator. Some of these products have been observed earlier in oxidation reactions with Cu(phen)$_2^+$.[10] Interestingly, products 4, 5, and 8 have, however, not been characterized previously, likely as a result of the alkaline treatments utilized. It is also noteworthy that the 2′-deoxyribonolactone (3) can still be observed 48 h after the photocleavage reaction and therefore seems to be a side product rather than an intermediate.

Photocleavage using [Rh(bpy)(phi)$_2$]$^{3+}$, which binds and cleaves mismatches but also canonical B-DNA, not surprisingly yields a wealth of peaks by MALDI-TOF analysis (Supporting Information). In addition to cleavage at the mismatched site, we see new products corresponding both to cleavage at alternate sites and also to cleavage at similar base positions as for **1** but with alternate masses. Based upon the absence of some products, it is likely that [Rh(bpy)(phi)$_2$]$^{3+}$

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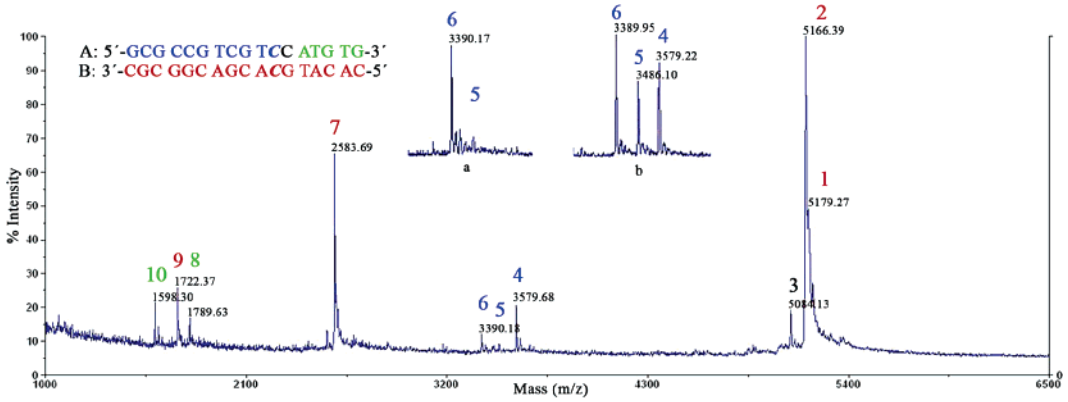

Figure 2. MALDI-TOF mass spectra obtained 40 min after photocleavage.[9] Cleavage products are numbered and described in the text. For the oligomer sequence shown, blue and green numbered products correspond, respectively, to cleavage fragments on the A strand 5′ and 3′ to the cleavage site, the **C** (in black) neighboring the mismatch; B strand products are in red. Inset a: MALDI-TOF mass spectra obtained later, 48 h after photocleavage with **1**. Inset b: MALDI-TOF mass spectra obtained 40 min after photocleavage with the parent **2** (2 μM), and 4 μM mismatched DNA.

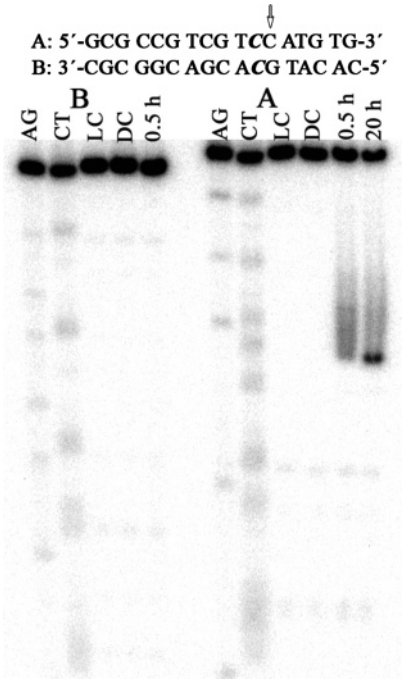

Figure 3. Phosphoimagery of a 20% polyacrylamide gel showing selective photocleavage on DNA strand A by **1**. For **A** and **B**, the A strand and B strand are 5′-^{32}P-end-labeled, respectively. For each series, AG and CT lanes are Maxam−Gilbert sequencing reactions; LC corresponds to samples irradiated but without metal complex; DC are samples with metal complex but no light, and 0.5 and 20 h correspond to times of incubation of samples after irradiation.[9] The mismatched DNA with marked mismatch and cleavage site is shown above.

cleaves differently than [Rh(bpy)$_2$chrysi]$^{3+}$. The many peaks are nonetheless a clear diagnostic of the lack of site specificity.

Overall, these data illustrate a new methodology to analyze DNA cleavage reactions by small molecules. The method is particularly useful in the analysis of molecules that cleave site specifically. The method requires no radioactive labeling, only little material, and analysis can be accomplished within minutes. Moreover, this mass spectral analysis of DNA cleavage yields direct information regarding products rather than simply the base pair site of cleavage.

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Supporting Information Available: Schemes outlining the synthesis of compound **1**, reaction products, and MALDI-TOF mass spectra of the control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089−1107. (b) Boerner, L. J. K.; Zaleski, J. M. *Curr. Opin. Chem. Biol.* **2005**, *9*, 135−144. (c) Erikkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777−2795.
(2) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303−2312.
(3) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731−735.
(4) (a) Jackson, B. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 12986−12987. (b) Jackson, B. A.; Alekseyev, V. Y.; Barton, J. K. *Biochemistry* **1999**, *38*, 4655−4662. (c) Jackson, B. A.; Barton, J. K. *Biochemistry* **2000**, *39*, 6176−6182. (d) Junicke, H.; Hart, J. R.; Kisko, J. L.; Glebov, O.; Kirsch, I. R.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3737−3742.
(5) Brunner, J.; Barton, J. K. Unpublished results.
(6) Banoub, J. H.; Newton, R. P.; Esmans, E.; Ewing, D. F.; Mackenzie, G. *Chem. Rev.* **2005**, *105*, 1869−1915.
(7) Dervan, P. B. *Science* **1986**, *232*, 464.
(8) Schatzschneider, U.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 8630−8631.
(9) Photocleavage conditions: 2 μM **1**, 2 μM mismatched DNA (for the sequence, see Figure 2), 50 mM NaCl, 10 mM NaPi pH 7, 15 min irradiation with a 1000 W Oriel Hg/Xe arc lamp and light of 320−440 nm wavelength.
(10) (a) Meijler, M. M.; Zelenke, O.; Sigman, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 1135−1136. (b) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107−1136.
(11) This product was proposed as an intermediate in the copper phenanthroline-mediated oxidative DNA cleavage. See: Chen, T.; Greenberg, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3815−3816.
(12) Degradation of metastable cleavage products over time was also observed by others. See: Kuwabara, M.; Yoon, C.; Goyne, T.; Thederahn, T.; Sigman, D. S. *Biochemistry* **1986**, *25*, 7401−7408.
(13) This higher cleavage yield as well as cleavage position with the unmodified complex is consistent with observations obtained by quantitative gel electrophoresis analysis of the photocleavage reaction.
(14) The products 4 and 8 as assigned would suggest that the phosphodiester backbone is cleaved prior to water attack.

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