VOLUME 23 NUMBER 9

ACCOUNTS OF CHEMICAL RESEARCH®

SEPTEMBER 1990

COMMENTARY

On Demonstrating DNA Intercalation

Considerable attention has focused on new DNA-binding and -modifying agents, from natural products to wholly synthetic designs, as probes of DNA structure and as potential chemotherapeutic agents.[1-4] The application of these molecules necessitates a precise understanding of the structural details of the agents' mode of interaction with the target molecule, double-helical DNA. DNA binding agents tend to interact noncovalently with the host molecule through two general modes: (i) in a groove-bound fashion stabilized by a mixture of hydrophobic, electrostatic, and hydrogen-bonding interactions and (ii) through an intercalative[5] association in which a planar, heteroaromatic moiety slides between the DNA base pairs. Surprisingly, however, only a fraction of known DNA-interactive agents have been structurally characterized to atomic detail in noncovalent complexes with DNA.[6,7]

Fortunately, numerous methods to probe the interaction of a DNA-binding molecule with its host in solution are available.[7,8] Given the diversity of methods used, however, it now becomes necessary to establish general experimental criteria for the classification of binding mode, in particular, as we address here, criteria that demonstrate intercalation of DNA-binding molecules in the absence of crystallographic determinations of complex structure. Setting these criteria is especially important in light of the synthesis of new "three-dimensional" intercalators in which the DNA-binding molecule may include but is not wholly described by a classical planar, aromatic structure. The intricate three-dimensional shape of such molecules provides opportunities for binding in several distinct orientations and modes along the helix. Therefore the question posited here arises: In the absence of an atomically resolved determination of structure, *what constitutes strong evidence for an intercalative interaction*?

Since its proposal by Lerman[5] in 1961 as a mode of DNA binding by planar aromatic molecules, the physical effects and characteristics of intercalation on DNA structure have been well studied. The following changes occur upon intercalation:

Unwinding and Lengthening of the DNA Helix. Intercalation produces an extension, unwinding, and stiffening of the DNA helix. These changes are a consequence of the untwisting of the base pairs and helical backbone needed to accommodate the intercalator.

Electronic Interaction of the Intercalator within the Helix. Intercalation results in an ordered stacking of the bound species between base pairs at 3.4 Å separation. The intercalating surface is sandwiched tightly between the aromatic, heterocyclic base pairs and stabilized[7] electronically in the helix by $\pi-\pi$ stacking and dipole–dipole interactions.

Rigidity and Orientation of the Intercalator within the DNA Helix. Upon intercalation at the unwound site, there is a substantial structural overlap between the base pairs and the intercalator. The intercalator becomes rigidly held and oriented with the planar moiety perpendicular to the helical axis.

The above description leads to minimal experimental criteria to establish intercalation:

1. Experiments That Evaluate Structural Changes in the DNA Helix. These involve the assessment of the changes in the helical or superhelical structure of DNA which occur upon binding, i.e., the "macromolecular" effects on the DNA induced by intercalation. Representative experiments include an assessment of changes in the solution viscosity of bulk DNA, or in the sedimentation coefficient or electrophoretic mobility of closed circular DNA caused by the lengthening and unwinding of the

(1) Barton, J. K. *Science* **1986**, *233*, 727. Fleisher, M. B.; Mei, H.-Y.; Barton, J. K. *Nucleic Acids Mol. Biol.* **1988**, *2*, 65. Barton, J. K.; Pyle, A. M. *Prog. Inorg. Chem.*, in press.
(2) Dervan, P. B. *Science* **1986**, *232*, 464. Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645.
(3) Chrisey, L. A.; Bonjar, G. H. S.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 644 and references therein. Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. *The Molecular Basis of Antibiotic Action*; Wiley: London, 1981. Zein, N.; Sinha, A. M.; McGarhren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198.
(4) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180. Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7368.
(5) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18.
(6) Kennard, O.; Hunter, W. N. *Q. Rev. Biophys.* **1989**, *22*, 327.
(7) Dougherty, G.; Pigram, W. J. *CRC Crit. Rev. Biochem.* **1982**, *12*, 103. Berman, H. M.; Young, P. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87.
(8) Waring, M. *J. Mol. Biol.* **1970**, *54*, 247.

0001-4842/90/0123-0271$02.50/0

Table I
Experimental Determinants of Intercalative Binding for Several DNA-Interactive Agents[a]

	DNA structural changes	electronic interaction	molecular orientation and rigidity	structural parameter	intercalation	ref
ethidium	+	+	+	+	+	6-8, 17
apoporphyrins	+	+	+	+	+	18
metalloporphyrins[b]	-	+	+	-	-	14
nonfused aromatics						
25° twist	+	+	+	+	+	15
48° twist	-	+	-	-	-	15
Pt(terpy)HET^{2+}	+	+	+	+	+	16
steroidal diamines	+	-	nd	-	-	8, 19
distamycin	-	+	-	-	-	20
bleomycin	+	+	nd	+/-	+/-	21
Ru(phen)$_3^{2+}$	+	+	+	+	+	13, 22, 24 25-27
Ru(TMP)$_3^{2+}$	-	-	-	-	-	23
DAPI						
GC sequences	+	+	+	+	+	28
AT sequences	-	-	-	+	-	28

[a] Key to symbols and abbreviations: nd, not determined; +, positive interaction; -, negative interaction; +/-, partial interaction. [b] Axially liganded porphyrins.

helix.[8,9] Downfield shifts in the ^{31}P NMR spectrum of the phosphodiester backbone of DNA are indicative also of the substantial structural perturbations in the sugar–phosphate backbone that accompany intercalation.[10]

2. Experiments That Indicate an Electronic Interaction between the Intercalator and DNA Bases. Binding commonly results in hypochromism and a shift to longer wavelength of the transition of the intercalated chromophore.[7] Emission enhancements (or quenching) are often observed in those molecules that luminesce, reflecting changes also in the excited state electronic structure. Also applicable[10] are ^{1}H NMR upfield shifts in the aromatic protons of the intercalated molecule which result from ring currents from the stacked aromatic bases.

3. Experiments That Demonstrate Molecular Orientation or Rigidity. These include dichroic techniques (linear flow[11] or electric[12]) where the bound orientation of the putative intercalator is evaluated relative to the oriented helical axis, and luminescence polarization experiments[13] which establish the time over which the molecule is rigidly bound within the helix.

4. Considerations of Molecular Shape and Structure. While the above experimental methodologies may point to an intercalative interaction, it is also incumbent upon the investigator to consider the structural ramifications in binding the putative intercalator to DNA. Researchers today are developing both synthetic and naturally occurring DNA-interactive molecules with functional domains that are planar but that in total may be far more complex in structure.[1,3,4] The challenge now becomes to determine fully and accurately the extent of intercalation in a molecule that may contain multiple binding modes and functional domains for binding. Thus additional structural criteria must become an integral part of any complete demonstration of intercalation. Examples of experiments that have directly addressed these structural aspects include the examination of the extent of intercalation of metalloporphyrins containing metals that either contain or lack the potential for axial ligation,[14] the systematic evaluation of the effect of molecular "twist" on intercalative parameters of nonfused aromatic molecules,[15] and the comparison using several binding assays of (bipyridyl)platinum(II) versus bis(pyridine)platinum(II) species, demonstrating a requirement for ligand planarity.[16]

Table I summarizes the conclusions from the application of experimental criteria 1–4 to several DNA-interactive agents. *The accumulated results suggest that the proposal or dismissal of an intercalative interaction cannot be concluded on the basis of any one of the above criteria.* The finding of hypochromism or fluorescence polarization alone, for example, is certainly insufficient to demonstrate intercalation. Often, as illustrated in Table I, nonintercalating groove-bound molecules can supply misleading results if selected but not all of the criteria are met. Misassignments may also arise if more than one binding mode is available to the binding agent depending upon environmental conditions or base composition.[28]

A pertinent example where all of the above criteria have

(9) Fisher, L. M.; Kuroda, R.; Sakai, T. T. *Biochemistry* **1985**, *24*, 3199.
(10) Gao, X.; Patel, D. J. *Q. Rev. Biophys.* **1989**, *22*, 93. Patel, D. J. *Acc. Chem. Res.* **1979**, *12*, 118. Marzilli, L. G.; Kline, T. P.; Live, D.; Zon, G. In *Metal-DNA Chemistry*; Tullius, T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989; p 119.
(11) Norden, B.; Tjernald, F. *Biophys. Chem.* **1976**, *4*, 191.
(12) Fritzsche, H.; Triebel, H.; Chaires, J. B.; Dattagupta, N.; Crothers, D. M. *Biochemistry* **1982**, *21*, 3940.
(13) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518.
(14) Pasternack, R. F.; Gibbs, E. J. In *Metal-DNA Chemistry*; Tullius, T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989; p 59. Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 2406, 5409. Geacintov, N. E.; Ibanez, V.; Rougee, M.; Bensasson, M. *Biochemistry* **1987**, *26*, 3087.
(15) Wilson, W. D.; Strekowski, L.; Tanious, F. A.; Watson, R. A.; Mokrosz, J. L.; Strekowska, A.; Webster, G. D.; Neidle, S. *J. Am. Chem. Soc.* **1988**, *110*, 8292.
(16) Lippard, S. J. *Acc. Chem. Res.* **1978**, *11*, 211.
(17) Bauer, W.; Vinograd, J. *J. Mol. Biol.* **1968**, *33*, 141.
(18) Fiel, R. J.; Beerman, T. A.; Mark, E. M.; Datta-Gupta, N. *Biochem. Biophys. Res. Commun.* **1983**, *107*, 1067.
(19) Mahler, H. R.; Baylor, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 256. Waring, M. J.; Chisolm, J. W. *Biochem. Biophys. Acta* **1972**, *262*, 18.
(20) Zimmer, C.; Wahnert, U. *Prog. Biophys. Mol. Biol.* **1986**, *47*, 31.
(21) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. Levy, M. J.; Hecht, S. M. *Biochemistry* **1988**, *27*, 2647.
(22) Barton, J. K.; Danishefsky, A. T.; Goldberg, J. M. *J. Am. Chem. Soc.* **1984**, *106*, 2172. Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 2081.
(23) Rehmann, J. P.; Barton, J. K. *Biochemistry* **1990**, *29*, 1701, 1710.
(24) Mei, H.-Y.; Barton, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 7414. Mei, H.-Y.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1339.
(25) Kelly, J. M.; Tossi, A. B.; McConnell, D. J.; OhUigin, C. *Nucleic Acids Res.* **1985**, *13*, 6017.
(26) Tossi, A. B.; Kelly, J. M. *Photochem. Photobiol.* **1989**, *49*, 545. Stadowski, C.; Gorner, H.; Currel, L. J.; Schulte-Frohlinde, D. *Biopolymers* **1987**, *26*, 189.
(27) Yamagishi, A. *J. Chem. Soc., Chem. Commun.* **1983**, 572.
(28) 4,6-Diamidino-2-phenylindole (DAPI) appears to groove-bind in AT-rich sequences and intercalate in GC sites. See: Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Strekowski, L.; Boykin, D. W.; Jones, R. L. *J. Am. Chem. Soc.* **1989**, *111*, 5008.

been examined in detail involves the binding of tris(phenanthroline)ruthenium(II), $Ru(phen)_3^{2+}$, to DNA.[13,22,23] Each of the three propeller-like bidentate ligands of the molecule provides a planar aromatic surface for potential intercalation, and together they impart chirality to the structure. NMR[23] and photophysical[22] experiments have established that two DNA-binding modes, intercalative and groove bound, are available to $Ru(phen)_3^{2+}$. This case, therefore, allows us to ask whether, in the presence of alternative binding modes, intercalation can be firmly established.

In fulfilling the first criterion for intercalation, $Ru(phen)_3^{2+}$ has been demonstrated to unwind helical DNA through two independent assays, one[22] measuring altered electrophoretic mobility, another[25] using a topoisomerase assay. The second criterion, establishing an electronic interaction between the potential intercalator and the DNA bases, has been firmly established through observations of hypochromism and red shifts in the MLCT band of the complex upon binding and based upon increases in the MLCT excited state lifetime.[13,22,26] A determination of rigid binding by $Ru(phen)_3^{2+}$, the third experimental criterion, has been established through emission polarization measurements that indicate the retention of polarization in emitted light.[13,22] In contrast, surface-bound molecules showed no retention of polarized emission.

The fourth criterion, a consideration of molecular shape and structure, is readily established through the comparison of Δ and Λ enantiomers with respect to each experimental determinant (structural changes, electronic interactions, and rigidity) for intercalative binding. In *all* of these experiments, the Δ enantiomer, at the same added concentration as the Λ isomer, shows an increased effect. This enantioselectivity is also supported by electric dichroism measurements.[27] The readily observed chiral discrimination provides perhaps the strongest evidence in support of intercalation based upon structural considerations and indicates that the symmetry of the metal complex matches the symmetry for *intercalation* into a right-handed helix.[1]

In contrast to these data, a recent publication[29] utilizing techniques (flow dichroism) that determine only the *orientation* of a bound species relative to the helix axis has described the construction of models for the interaction of $Ru(phen)_3^{2+}$ with DNA, models in which intercalation was not predominant. The orientational data alone were clearly incapable of resolving the two binding modes for the complex as established by several different lines of evidence.[22,23,26] Clearly, the above line of experimentation provides an example of how the application of any one of the four criteria *alone* may lead to ambiguities in characterizing the binding modes of a molecule.[30]

In summary, to assess the binding interactions with DNA of "three-dimensional" DNA-interactive molecules in the absence of a well-resolved crystal structure, what is required is an evaluation that satisfies at least four criteria prior to establishing an intercalative interaction. Hopefully these criteria will set a standard by which other workers in the field may judge their data during the evaluation of binding mode and aid in the future rational design of DNA-interactive agents.

We are grateful to the National Institutes of Health for their support of our work. E.C.L. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

(29) Hiort, C.; Norden, B.; Rodger, A. *J. Am. Chem. Soc.* **1990**, *112*, 1971.

(30) It is noteworthy that none of the above experimental criteria speak to the question of the groove location (minor versus major) of a DNA-binding agent, and here as well experimental evidence is required.

* To whom correspondence should be addressed.

Eric C. Long and Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California 91125

ARTICLES

Ketene Chemistry: The Second Golden Age

THOMAS T. TIDWELL

Department of Chemistry, University of Toronto, Scarborough Campus, Scarborough, Ontario, Canada M1C 1A4

Received October 31, 1989 (Revised Manuscript Received June 6, 1990)

A decade ago our group first began to examine the reaction mechanisms[1a] and synthetic applications[1b] of nucleophilic additions to ketenes. It appeared to us that this field had not received the attention warranted by the intrinsic interest and potential utility of these fascinating difunctional species. This area of study reached its first maturity very early, as it was the initial major research interest of Hermann Staudinger, who reported the first ketene in 1905. He summarized his work on the subject in a definitive monograph in 1912,[2] which marked the culmination of the first golden age of ketene chemistry. Staudinger then went on to develop further interests, such as the creation of modern polymer chemistry.

The field did not languish thereafter, but received steady attention by some talented groups as summarized in several reviews,[3] but only after Woodward and

Born in 1939, Thomas Tidwell received his B.Sc. at Georgia Tech and his Ph.D. with Paul Bartlett at Harvard in 1964. He is presently Professor of Chemistry at the University of Toronto, where his main research interests lie in the areas of ketene and organofluorine chemistry.

(1) (a) Kabir, S. H.; Seikaly, H. R.; Tidwell, T. T. *J. Am. Chem. Soc.* **1979**, *101*, 1059–1060. (b) Tidwell, T. T. *Tetrahedron Lett.* **1979**, 4615–4618.

(2) Staudinger, H. *Die Ketene*; Enke: Stuttgart, 1912. For an autobiographical account, see: Staudinger, H. *From Organic Chemistry to Macromolecules*; Wiley: New York, 1970.

(3) (a) *The Chemistry of Ketenes, Allenes and Related Compounds*; Patai, S., Ed.; Wiley: New York, 1980; Parts 1 and 2. (b) Brady, W. T. *Tetrahedron* **1981**, *37*, 2949–2966. (c) Borrmann, D. *Methoden der Organische Chemie*; Thieme: Stuttgart, 1968; Vol. 7, Part 4.

0001-4842/90/0123-0273$02.50/0