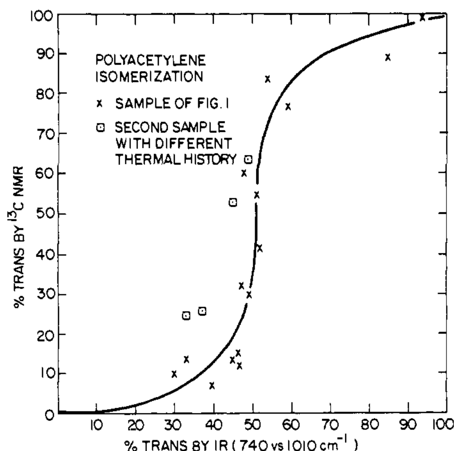

Figure 2. Percent polyacetylene trans isomer as calculated from integrated peak areas in the NMR spectra vs. trans content determined from absorbances of the 1010- (trans) and 740-cm^{-1} (cis) bands in the IR spectra.[8]

Polyacetylene was prepared by the Shirakawa technique[12] and stored at -80 °C. Samples for IR and NMR spectroscopy were handled in an oxygen-free (<0.8 ppm) glove box under argon. Cis/trans content was determined by using the 740- ("*cis*") and 1010-cm^{-1} ("trans") IR bands.[8] A sample initially 30% trans by IR analysis was monitored at 25 °C for 11 days as it became 47% trans by IR analysis. The sample was then treated incrementally at 100 °C until was 85% trans by IR spectroscopy and then at 200 °C to yield 94% trans by IR analysis.

The NMR spectra for this series of isomerization experiments are shown in Figure 1. The cis peak appears at 128.5 ± 0.4 ppm and the trans at 137.3 ± 0.4 ppm downfield from Me_4Si. Note that the trans signal is nearly absent from the initial 30% trans (IR) sample. Even after 9 days at 25 °C the 47% trans (IR) sample shows only a weak trans NMR signal. But upon isomerization at higher temperature (100 °C), the trans NMR peak does appear, weak at first but increasing in intensity, in spite of the fact that IR analysis reveals no meaningful change in composition. This is graphically illustrated in Figure 2; in the range where NMR indicates a change from 15% to 85% trans, IR shows very little change (45% to 55% *trans*).

The discrepancy in cis/trans ratios determined by NMR peak integration and conventional IR analysis can be rationalized by considering (i) the IR analysis to be in error because of sequence length effects upon the apparent relative extinction coefficients of the cis (**1**) and trans (**3**) peaks,[13] (ii) the downfield NMR peak, assigned to the trans isomer, to be due solely to triad or longer trans sequences, (iii) the existence of a third isomer, the trans-cisoid (**2**),[14] those NMR chemical shift is nearly the same as that of the cis peak, or (iv) the polymer crystalline and amorphous regions to have different isomerization rates[15] and nonquantitative relative ^{13}C peak intensities.[16] More detailed work is in progress to differentiate these possibilities.

It is noteworthy that the thermal history of the polyacetylene affects the relationship between the IR and NMR results as shown in Figure 2 for a second independent sample. This suggests that one or more of the aforementioned structural features is/are dependent upon thermal history. Thus, carefully monitored sample histories are crucial to detailed understanding of the microstructure of polyacetylene and, by inference, its physical and chemical properties.

These findings clearly demonstrate the inadequacy of IR determination of composition and the importance of sample history. While these results underscore the complexity of polyacetylene, they provide the basis for understanding a number of conflicting electrical, magnetic, and spectroscopic measurements reported for this interesting polymer.

(12) Ito, T.; Shirakawa, H.; Ikeda, S. *J. Polym. Sci., Polym. Chem. Ed.* **1974**, *12*, 11.
(13) For the doubling of a *trans*-polyene absorption in the presence of a cis linkage in carotene, see, e.g.: Nakanishi, K. "Infrared Absorption Spectroscopy"; Holden Day: San Francisco, 1962; p 125. Also see: Lunde, K.; Zechmeister, L. *J. Am. Chem. Soc.* **1955**, *77*, 1647. Various sequence distributions could lead to multiple absorptions which would manifest broadening and decreased intensity (absorbance). On this basis we favor use of integrated peak areas for isomeric composition determination.
(14) The calculated total energies of isomers **1** and **2** are very similar: 1.9 and 2.1 kcal per C_2H_2 unit, respectively, relative to **3** (Karpfen, A.; Holler, R. *Solid State Commun.* **1980**, *37*, 179. This argument would require that in the IR spectra both **2** and **3** appear at ca. 1010 cm^{-1}, in contrast to the normal mode analysis of Shirakawa and Ikeda (Shirakawa, H.; Ikeda, S. *Polym. J.* **1971**, *2*, 231).
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cis-Diamminedichloroplatinum(II) Binds in a Unique Manner to Oligo(dG)·Oligo(dC) Sequences in DNA—A New Assay Using Exonuclease III

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Previous experiments[1] suggested that the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) binds in a sequence specific manner to DNA. In particular, low levels of bound *cis*-DDP selectively inhibit the restriction endonuclease Pst I from cutting plasmid pSM1 at a site with an adjacent $(dG)_4$·$(dC)_4$ sequence. This sequence was noteworthy since binding of *cis*-DDP to poly(dG)·poly(dC) produces substantially greater buoyant density increases than binding to poly(dG·dC).[2] Here we demonstrate more directly that *cis*-DDP binds in a unique fashion to $(dG)_n$·$(dC)_n$, $n \geq 2$, sequences in DNA through a new assay by using exonuclease III in conjunction with DNA sequencing techniques.

Both the Maxam–Gilbert[3] and Sanger[4] methods of rapid DNA sequencing employ a strategy whereby the position and type of base in the sequence of a DNA molecule are determined by the distance of that base from one end of the chain. This determination is achieved by constructing a set of fragments of the DNA molecule whose sequence is desired. These fragments have two important characteristics. The first is that they all have one end in common. This requirement is assured in the Maxam–Gilbert method by radioactively labeling one end of one strand of the DNA duplex. The second characteristic of the fragments is that their other ends occur only at the positions of one of the four nucleotide bases. The Maxam–Gilbert technique accomplishes this objective by means of four chemically different sets of reactions, each of which specifically breaks the phosphodiester backbone of the DNA molecule at one type of base.

For a visualization of the sequence, the products of these four base-specific reactions are run in parallel on a high-resolution electrophoresis gel which is capable of separating DNA molecules differing in length by only one base. Autoradiography of the gel reveals the positions of the radioactively labeled fragments. The distance of migration of each molecule is proportional to its length. From the chemical reaction used to cleave the DNA chain to produce a fragment of a specific length, one may identify the base occurring at that position in the sequence. We have employed

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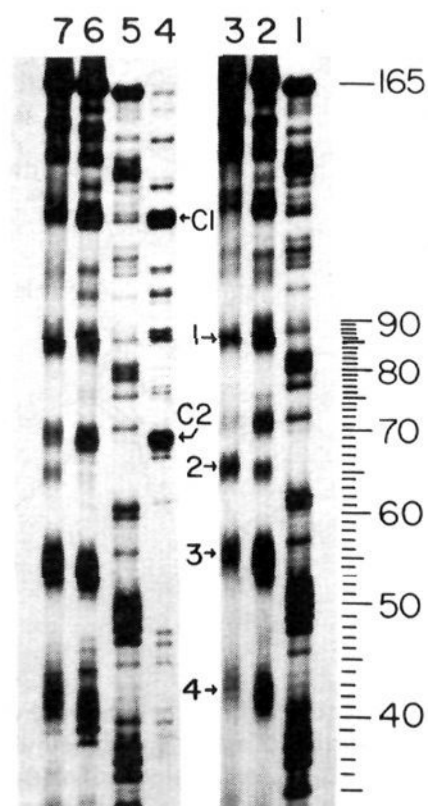

Figure 1. Autoradiograph of an 8% polyacrylamide/7 M urea electrophoresis gel showing the results of exonuclease III digestion of ^{32}P end labeled 165 bp DNA. Lanes 1 and 5, Maxam–Gilbert G-specific sequencing reaction products. Lanes 2 and 3, products of exonuclease III digestion of platinated DNA, with platinum removed by NaCN treatment before electrophoresis. Lane 4, products of exonuclease III digestion of unplatinated DNA. Lanes 6 and 7, same as lanes 2 and 3 except that platinated DNA was treated with NaCN to remove platinum before exonuclease III digestion. Lanes 2 and 6, $r_f = 0.05$. Lanes 3 and 7, $r_f = 0.10$. Bands are labeled as described in the text. The sequence of bases 35–90 on this strand[5] is 5′-GA*GGGA*$_{40}$GCTTCCA*GGG*$_{50}$*GG*AAACGCCT$_{60}$*GG*TATCTTTA$_{70}$-TAGTCCTGTC$_{80}$*GGG*TTTCGCC-3′$_{90}$. Italicized bases correspond to the oligo(dG) sites shown in lane 1 of the figure.

this strategy of relating the length of an end-labeled DNA molecule to the identity of its terminal base to determine the *cis*-DDP binding sites.

For these experiments we used a 165 base pair (bp) long DNA molecule containing a variety of $(dG)_n$ sequences, $n = 1, 2, 3, 5, 6$, on one strand. This molecule was obtained from pBR322, a bacterial plasmid of known sequence,[5] by the successive action of restriction endonucleases Hpa II and Hae III. This restriction fragment, labeled at the 5′ end with ^{32}P on the strand containing the oligo(dG) sites,[6] was incubated with *cis*-DDP in 1 mM sodium phosphate buffer, pH 7.4, 3 mM NaCl, at 37 °C for 3 h.[7] The formal ratio (r_f) of Pt to DNA phosphate, 0.05 or 0.1, was calculated relative to the amount of carrier salmon sperm DNA[8] in the reaction, usually 1 μg. From previous studies of *cis*-DDP binding to pBR322 DNA,[9] we estimate that, on the average, ~8 or ~16 Pt atoms, respectively, would be bound to the 165 bp DNA molecule under these conditions.

(5) Sutcliffe, J. G. *Cold Spring Harbor Symp. Quant. Biol.* **1979**, *43*, 77–90.

(6) Plasmid pBR322 was first digested with Hpa II (Bethesda Research Laboratories), and the second largest restriction fragment (527 bp) was isolated from a preparative low melting agarose electrophoresis gel. Terminal phosphates were removed by treatment with bacterial alkaline phosphatase (Bethesda Research Laboratories). The 5′ ends were labeled with ^{32}P by using T4 polynucleotide kinase (Bethesda Research Laboratories) and [γ-^{32}P]ATP (Amersham). This doubly end-labeled molecule was digested with Hae III (Bethesda Research Laboratories) and the singly end-labeled 165 bp fragment was isolated by electrophoresis on a 6% polyacrylamide gel. This DNA molecule, end labeled on the strand which contains the oligo(dG) sites, is suitable for analysis by the Maxam–Gilbert sequencing method.[3] We used the ladders generated by electrophoresis of the sequencing reaction products as a "ruler" to pinpoint the sequences where *cis*-DDP affects exonuclease III digestion of the labeled 165 base strand.

(7) Cohen, G. L.; Bauer, W. R.; Barton, J. K.; Lippard, S. J. *Science (Washington, D.C.)* **1979**, *203*, 1014–1016.

(8) A large excess of unlabeled (carrier) DNA is present to aid in precipitation and isolation of the small amount of labeled DNA.

(9) Ushay, H. M.; Tullius, T. D.; Lippard, S. J. *Biochemistry*, in press.

Exonuclease III is an enzyme that degrades duplex DNA exonucleolytically from the 3′ end and nicks DNA at apurinic and apyrimidinic sites.[10] The enzyme produces two major sets of radioactive products when it digests the labeled unplatinated 165 bp DNA molecule under our conditions.[11] Two sets of intense bands, a triplet of 115–117 bases (band C1) and a doublet of 71 and 72 bases (band C2), are seen[12] (Figure 1, lane 4) when the reaction products are denatured and separated by electrophoresis on an 8% polyacrylamide/7 M urea thin DNA sequencing gel.[13]

Exonuclease III digestion of the platinated DNA produces DNA molecules shorter than 165 bases which migrate as discrete, albeit smeared, bands upon electrophoresis. The bands sharpen slightly if the DNA is treated with 0.2 M NaCN for 3 h at 37 °C to remove bound platinum[14] before electrophoresis (Figure 1, lanes 2 and 3). Some of the labeled DNA runs as a diffuse smear at the top of the gel (not shown) even with exonuclease III and cyanide treatment. At low levels of bound *cis*-DDP, bands corresponding to the major products of exonuclease III digestion of unplatinated DNA are still evident. These bands run with slightly lower mobility than bands from unplatinated samples, presumably due to the charge effect of bound platinum.[15] In addition, several new bands (Figure 1, lane 2, bands 1–4) appear. With increasing amounts of bound *cis*-DDP the "normal" exonuclease III products disappear while the new bands remain (Figure 1, lane 3).

Comparison of the positions of bands 1–4 to the positions of marker fragments produced by the Maxam–Gilbert sequencing reactions[16] show that they are not in register with any common sequence on the DNA. Closer inspection, however, reveals that bands 1–4 have comigrated with DNA molecules uniformly four bases longer than marker fragments corresponding to oligo(dG) sites (Figure 1, lanes 1 and 5). Since the products of the chemical sequencing reaction lack the base with which they are identified,[16] there is apparently[17] a three-base shift of the mobilities of the exonuclease III fragments relative to the positions expected if exonuclease III stopped digesting the DNA precisely at platinated oligo(dG)·oligo(dC) sites. Bands 1–4 are therefore attributed to exonuclease III digestion that terminates three bases to the 3′ direction of an oligo(dG)·oligo(dC) site containing bound *cis*-DDP. These bands are in a region of the gel with very high resolution so that the three-base shift can be easily measured. Isolated G·C base pairs in this region do not give rise to new bands within the limits of detection of our experiment. Exonuclease III does not produce bands 1–4 upon digestion of DNA platinated with

(10) Rogers, S. G.; Weiss, B. *Methods Enzymol.* **1980**, *65*, 201–211.

(11) Exonuclease III (Bethesda Research Laboratories) reactions were carried out in 50 mM Tris-Cl buffer (pH 8.0), 10 mM 2-mercaptoethanol, and 0.66 mM $MgCl_2$, in a volume of 0.050 mL. Forty units of exonuclease III were used for each 1 μg of carrier DNA. A rough calculation, on the basis of published specific activity of purified exonuclease III (162 000 units/mg, ref 10), its M_r (28 000, ref 10), and the number of carrier DNA 3′ ends in our reaction, showed that exonuclease III was in approximately 3-fold excess over the number of 3′ ends. Reactions were run for 1 h at 37 °C and stopped by addition of EDTA to 2 mM and phenol extraction. 2-Mercaptoethanol will not remove *cis*-DDP from DNA when incubated under these conditions (ref 9).

(12) Many other bands, of much lower intensity, are also observed. These mainly consist of DNA molecules of length greater than 70 bases. Few bands are seen due to molecules shorter than 70 bases; so this region of the gel offers an especially clear background on which to observe new bands resulting from exonuclease III digestion of platinated DNA.

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(14) Bauer, W. R.; Gonias, S. L.; Kam, S. K.; Wu, K. C.; Lippard, S. J. *Biochemistry* **1978**, *17*, 1060–1068.

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(17) This apparent shift could be due in part to the charge effect of bound platinum.[15] As seen for bands C1 and C2 in platinated samples (vide supra), platination could account for a one- or two-base shift. The rest of the shift might occur if *cis*-DDP were to denature the DNA a few bases on either side of its binding site,[7] thereby disrupting the base pairing required for exonuclease III activity. Alternatively, the site on exonuclease III that recognizes the suitability of a DNA substrate might be distant from the cutting site of the enzyme. If a platinated base were an unsuitable substrate, the enzyme would stop cutting before it reached the platinated base. We are investigating these questions.

trans-DDP or [(dien)PtCl]Cl,[18] neither of which has antitumor activity.

An experiment was performed in which platinated DNA was treated with cyanide before exonuclease III digestion to see whether irreversible lesions were induced in the DNA by *cis*-DDP (Figure 1, lanes 6 and 7). Most of the major bands seen in control digests of unplatinated DNA were restored by the cyanide pretreatment. The bands near the $(dG)_5{\cdot}(dC)_5$ and the $(dG)_3{\cdot}(dC)_3$ sites (bands 1, 3 and 4) were still present, however, even when most of the platinum was removed before exonuclease III digestion. This result is reminiscent of previous observations[14] of a fraction of *cis*-DDP bound to DNA that could not be removed by cyanide treatment.

In summary, *cis*-DDP bound to $(dG)_n{\cdot}(dC)_n$ sequences, $n \geq 2$, modifies in a characteristic way the digestion of DNA by exonuclease III. This is the most direct demonstration to date of sequence specificity of the mode of binding of *cis*-DDP to DNA. The results are consistent with models that postulate binding of *cis*-DDP to adjacent deoxyquanosines or deoxycytidines as the effective lesion in platinum chemotherapy. Oligo(dG)·oligo(dC) sequences should be a major focus of attention for chemists engaged in modeling the interactions of *cis*-DDP with DNA.

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Stereoelectronic Properties of Photosynthetic and Related Systems. 10. Quantum Mechanical Characterization of the Excited States of Ethyl Chlorophyllide *a* Enol

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The idea that enolic tautomers of chlorophyll *a* (Chl *a*) might participate as intermediates in the primary events of photosynthesis in photosystem I of green plants has been postulated[1,2] but has received little or no experimental verification. Indeed, there has been no experimental characterization of Chl *a* enols until recently, when the optical and NMR spectra of Chl *a* silyl enol ethers[3] and related systems[4] were reported. More significantly, however, Wasielewski et al.[5] have examined the redox properties of Chl enols II and III (see Figure 1) as well as the magnetic resonance (ESR, ENDOR) spectra of the cation radicals of these systems. On the basis of their data they concluded that the π-cation radical of monomeric Chl *a* enol may be a more plausible alternative as a model for P700+, the oxidized phototrap of photosystem I, than is the Chl *a* "special pair" dimer model proposed earlier.[6-8]

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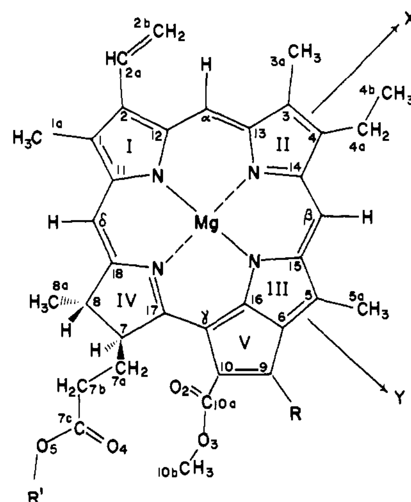

Figure 1. Molecular structure of (I) ethyl chlorophyllide *a* enol (R = OH, R′ = C_2H_5); (II) chlorophyll *a* *tert*-butyldimethylsilyl enol ether (R = OSi-*t*-$BuMe_2$, R′ = $C_{20}H_{39}$); (III) 9-deoxo-10-dehydro-9-hydrochlorophyll *a* (R = H, R′ = $C_{20}H_{39}$). EtChl *a* (keto form) is obtained from I by reduction of the C_9–C_{10} double bond, addition of H at position 10, and substitution of a carbonyl oxygen for R at position 9.

Table I. Estimated Transition Energies, ΔE^{est}, Magnitudes of the Electric Dipole Moment, $|\mu|$, and Vector Components of the Dipole Moment for Low-Lying States of EtChl *a* Enol and EtChl *a*

state	ΔE^{est}, cm^{-1} [a]	$\lvert\mu\rvert$, D	μ_x,[b] D	μ_y,[b] D	μ_z,[b] D
		EtChl *a* Enol			
S_0	0.0	5.46	−3.86	2.35	3.06
S_1	9922	9.68	−6.67	6.38	2.91
S_2	15 285	8.17	−5.80	4.87	3.05
S_3	18 109	9.30	−6.02	6.42	2.99
T_1	8111	7.79	−5.11	5.07	2.98
T_2	12 733	8.28	−5.78	5.11	3.03
T_3	14 968	9.23	−7.13	5.02	3.03
		EtChl *a*[c]			
S_0	0.0	3.43	−1.62	−0.353	3.00
S_1	15 810	3.56	−1.79	0.811	2.97
T_1	10 740	3.31	−1.17	0.995	2.93

[a] Obtained from the equation given in ref 16. [b] A positive vector component points along the indicated axis from the origin of Figure 1 toward a region of positive charge. [c] Data computed from wave functions reported in ref 12.

The present communication deals with the interpretation of several important features of the electronic absorption spectra of Chl *a* enols. For this purpose, ab initio configuration interaction (CI) calculations were performed on ethyl chlorophyllide *a* enol (EtChl *a* enol, compound I in Figure 1), to obtain a theoretical description of the ground and low-lying singlet and triplet excited states. The computations performed include a self-consistent-field (SCF) calculation on the molecular ground state[9] using a basis set of floating spherical Gaussian orbitals (FSGO),[10] followed by the calculation of ground- and excited-state CI wave functions utilizing the CI procedure of Whitten and Hackmeyer.[11] Com-

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(9) Ring V (see Figure 1) was taken to be essentially planar. The C_9–C_{10} bond was taken to be the average of the ββ′ carbon–carbon bond lengths of the individual pyrroles of rings I, II, and III, and the Cγ–C_{10} and C_6–C_9 bonds were taken to be the average of the αβ′ and α′β′ carbon–carbon bond lengths of the individual pyrroles of rings I, II, and III. The remainder of the geometry was taken from the X-ray crystal structure of EtChl *a*·$2H_2O$: Chow, H.-C.; Serlin, R.; Strouse, C. E. *J. Am. Chem. Soc.* **1975**, *97*, 7230–7237.
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