organic solvent with a moderately high dielectric. Some care was taken to remove trace water from acetonitrile by refluxing with phosphorus pentoxide for 1-1.5 h at 82 °C. The thermodynamics of tubule formation in dry acetonitrile was investigated by using differential scanning calorimetry (DSC). Following thermal cycling of the acetonitrile-lipid suspension, the DSC pan was opened at room temperature and the sample examined with light microscopy. Parallel experiments were performed in a temperature bath and on a temperature controlled microscope stage.

Figure 1 shows the calorimetric result of heating and cooling the lipid-acetonitrile suspension. A transition temperature of 42.5 °C is observed upon heating with an enthalpy of 17.8 kcal/mol. Cooling scans reveal a transition at 31.5 °C with an enthalpy of 18.9 kcal/mol. The enthalpy values for these same transitions previously examined in water depend somewhat on the thermal history of the sample[7,11,12] but generally are 3-5 kcal/mol higher than those observed in this study. Parts A and B of Figure 2 show light micrographs of tubules formed in the DSC pan and on the thermal stage of the microscope, respectively. These tubules have similar macroscopic dimensions to those tubules formed in water (10-50 μm in length, 0.5μm in diameter). The conversion to tubules is not complete, with some precipitated lipid present in a nontubular form, which may account for the difference in enthalpy values observed in dry acetonitrile and water. Spherulites and focal conic domains also appear under cross polarization in the samples cooled on the thermal stage and from the DSC pan (Figure 2C,D). The spherulites show a characteristic pattern (maltese cross) under cross polarization, which may be similar to the patterns commonly observed in the smectic phase of other liquid crystals.[10] The observed pattern in the spherulites may indicate that the crystalline form may have uniaxial symmetry. The observation of spherulites and focal conic domains reveals the tendency of this lipid to self-assemble into lamellar structures in the absence of water. The spherulites may be similar to a low-temperature phase observed in the diacetylenic lecithins in water which appears in freeze fracture replicas as stacked lamellar sheets.[11]

Although we have taken great care to minimize the amount of water present in these experiments, it is possible that some residual water remains (e.g., the water of hydration of the lipid) and that the dry lipid itself is not completely anhydrous. It is significant, however, that self-assembly of tubules occurs under these dramatically reduced water conditions. The two previously reported methods of tubule formation rely on the presence of bulk water.[8,12,13] One of these methods involves the isothermal precipitation of tubules from ethanol-water solution.[14] Spectroscopic characterization and X-ray diffraction analysis of tubules reveal the presence of highly crystalline acyl chains.[14,15] The crystalline nature of the tubules and the observations presented in this work suggest that bulk water may not play a major role in the self-assembly of tubules. This is in contrast to the major role water does have in the formation of other self-assemblies formed from biologically derived amphiphiles. It may be that the dielectric of acetonitrile is sufficient to drive the assembly of this chemically modified phospholipid. We are continuing our examination of tubule formation and what conditions govern the formation of the spherulites to further understand forces that drive the self-assembly of this lipid.

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Efficient, Specific Cross-Linking and Cleavage of DNA by Stable, Synthetic Complementary Oligodeoxynucleotides[1]

Rich B. Meyer, Jr.,* John C. Tabone, Gerald D. Hurst, Todd M. Smith, and Howard Gamper

MicroProbe Corporation, 1725 220th Street SE Bothell, Washington 98021

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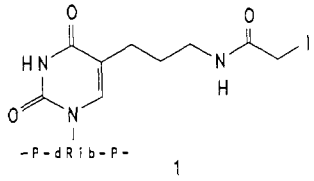
"Antisense" oligodeoxynucleotides (ODNs) have generated significant recent interest as potential chemotherapeutic agents.[2] Despite theoretical appeal, the potency necessary for animal or clinical trials has not yet been shown. We have been investigating the attachment of a side chain, bearing an alkylating electrophile, to ODNs as a method of achieving irreversible inactivation of the complementary target nucleic acid.[3] These ODNs can, as shown here, function as exceptionally selective cleavage reagents for DNA.

In their pioneering work, Brookes and Lawley[4] proposed that a five-atom interstrand cross-link was formed by bis(2-chloroethyl)amines between the N-7 of guanines in adjacent base pairs of GC sequences. Using molecular models of B form DNA, we found that a five- or six-atom arm should diagonally bridge the major groove from the 5-position of a pyrimidine in an ODN to the N-7 of a purine paired to the residue on the 3'-side of the pyrimidine bearing the arm.



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To test our model, we prepared a tetradecadeoxynucleotide (ODN-I), incorporating 5-[3-(iodoacetamido)propyl]-2'-deoxyuridine (**1**) in place of a T, and a 30-mer containing the sequence complementary to ODN-I using a sequence derived from human papillomavirus (HPV) type 16, as shown below. Nucleoside **1** has the requisite six-atom chain to bridge the major groove on to the nucleophilic guanine (G-21) in the target strand.

```
                          5     10    15    20    25    30
                          |     |     |     |     |     |
Target:      5'-AGA CAG CAC AGA ATT CGC AGG AAC ATC CAG-3'
ODN-I:                                3'-CG TCC ITG TAG GTC-5'
```

5-[3-(Trifluoroacetamido)propyl]-2'-deoxyuridine[5] was converted to the 5'-*O*-dimethoxytrityl-3'-(cyanoethyl *N*,*N*-diisopropylphosphoramidite) derivative by standard methods[6] for direct use on an automated DNA synthesizer.[7] The deprotected ODN[8]

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(8) After removal of the oligonucleotide blocking groups by ammonolysis, the ODN was purified by reverse-phase HPLC and detritylated with 80% acetic acid. Desalting was accomplished on a G-25 Sephadex column, followed by concentration.

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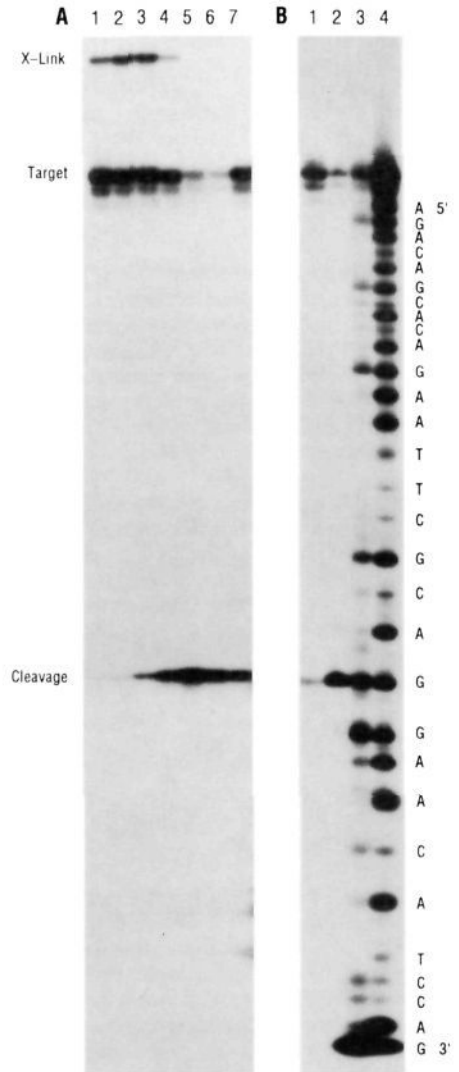

Figure 1. (A) Extent of crosslinking after 48 h at various temperatures. Each reaction contains 1 μg of ODN-I and 0.01 μg of ^{32}P-labeled cordycepin-tailed target in 100 μL of 10 mM phosphate (pH 7.2), 150 mM NaCl. Aliquots of 10 μL were removed at 48 h, quenched with 10 μL of 10 mM mercaptoethylamine, and analyzed by electrophoresis on a 7 M urea 20% polyacrylamide gel. Lanes 1–7: incubation at 23, 30, 37, 44, 51, 58, and 65 °C, respectively. (B) Specificity of the cross-linking reaction. The reactions were prepared as above and were incubated at 37 °C for 12 days. The following additions were made. Lane 1: 10 μg of ODN-I analogue lacking the cross-linking arm. Lane 2: 10 μg of noncomplementary 24-mer oligonucleotide. Lanes 3 and 4: Maxam–Gilbert G and G+A sequence ladders, respectively.

containing 5-(3-aminopropyl)-2′-deoxyuridine was iodoacetylated[9] with *N*-hydroxysuccinimidyl iodoacetate. The cross-linking reaction was studied by incubating ^{32}P-labeled[10] target with excess probe at various temperatures and then quenching with 10 mM mercaptoethylamine for subsequent analysis.

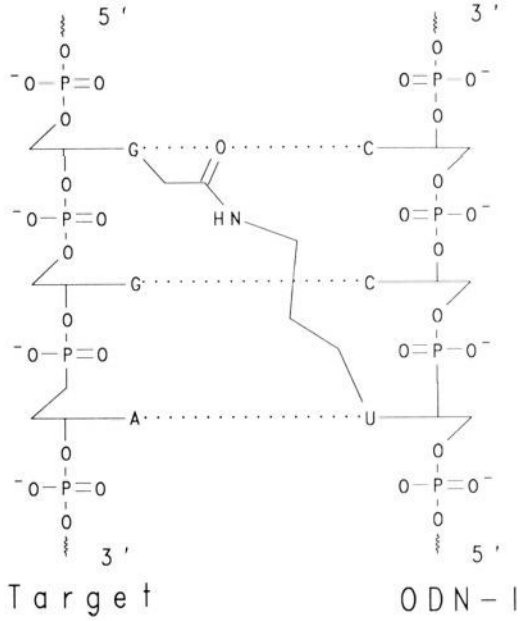

Figure 2.

In this system the extent of cross-linkage increased with both temperature and time. Figure 1A shows an autoradiograph of the electrophoretic pattern obtained after 48-h incubation at various temperatures. An apparent single higher molecular weight band appears for covalently cross-linked material. The rate of cross-linking shows an increase with temperature up to 37 °C, after which a low molecular weight species increases in intensity. This is the undecamer resulting from the four-step process of (a) hybridization, (b) cross-linkage of the hybrid, (c) depurination of the alkylated guanine, and (d) scission of the target strand at the depurination site. Total target strand modification[11] peaks at 51 °C, a few degrees above the estimated[12] T_m for the hybrid of 44 °C. The decrease in cleaved product at temperatures above the T_m strongly supports the suggestion that alkylation (cross-linking) only occurs within a base-paired hybrid. Consistent with this interpretation, cross-linking is inhibited in the presence of an excess of a competing ODN-I analogue lacking the electrophilic side arm (see lane 1, Figure 1B).[13] When ^{32}P-labeled ODN-I was incubated (37 °C, 2 days) with excess tRNA, β-galactosidase, or salmon sperm DNA, *no* nonspecific alkylation of these macromolecules was seen (data not shown).[14]

To elucidate the site of alkylation, the cross-linked HPV hybrid was cleaved at alkylated or depurinated strands 3′ to the site of alkylation by the Maxam–Gilbert piperidine method[15] or by prolonged incubation at 37 °C. Surprisingly, analysis of the cleaved product on a sequencing gel (see Figure 1B) indicates that the target strand guanine G-20, which is *two* base pairs removed from **1**, was the exclusive site of alkylation, as shown schematically in Figure 2. Originally, the anticipated cross-link site was a 5′-GA-3′ sequence in the target, in analogy with the Brookes and Lawley hypothesis.[16] Our target sequence, therefore, for an ODN

(9) Excess *N*-hydroxysuccinimidyl iodoacetate in borate buffer, pH 8.3, was added at room temperature and mixed. After 30 min, the derivatized ODN (ODN-I) was purified by desalting on a Sephadex G-25 column. Analytical HPLC using a Zorbax column (DuPont) gave adequate resolution when a 60–80% gradient of MeCN/0.02 N NaH_2PO_4 (4:1) to 1 N NaCl in MeCN/0.02 N NaH_2PO_4 (4:1) was used. Retention times were as follows: unsubstituted 14-mer, 9.81 min; aminopropyl 14-mer, 7.36 min; ODN-I, 10.09 min. The presence of the iodoacetyl moiety was confirmed by treatment with a 1.0 N solution of 2-aminoethanethiol, which adds one positive charge to the ODN. The elution time returned to that of the aminopropyl ODN.

(10) Target ODN was ^{32}P-labeled by cordycepin tailing with terminal transferase: Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning. A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1982; p 239.

(11) The sum of cross-linked hybrid, apurinic hybrid, apurinic target, and cleaved target.

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(13) The amount of cleavage in the presence of the competitor is <10% by densitometry.

(14) ODN-I was ^{32}P-labeled on the 5′-end by using polynucleotide kinase: Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning. A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1982; p 122.

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(16) Preliminary computer-assisted molecular modeling studies suggest that the amide linkage in the side arom of ODN-I forms a hydrogen bond with the cytosine on its 3′-side, an interaction that firmly orients the electrophilic site on the side arm toward the "second" guanine in the target.

containing **1**, should contain 5′-GXA-3′, and the cross-linking ODN should contain 3′-CY**1**-5′, where X and Y are complementary nucleotides.[17]

Several observations can be made regarding the significance of these results. First, the cleavage at 51 or 58 °C after 48 h is virtually quantitiative while absolute specificity is retained, making this method appropriate for the sequence-specific cleavage of single-stranded DNA. Second, the demonstrated site of alkylation suggests that the original Brookes and Lawley hypothesis regarding cross-linking across GC doublets may require revision; perhaps the actual cross-link is from G to G in GXC triplets. Third, the ability of the modified ODN to selectively alkylate the desired target without random alkylation on noncomplementary nucleic acids supports their use in chemotherapeutic agents if the cross-linking rate can be improved.

(17) To examine the regiospecificity of the reaction, we prepared a 24-mer target containing the sequence 5′-CGA-3′ and a cross-linking 14-mer ODN containing 3′-GC**1**-5′. No cross-linked or cleaved target strand was found when these were incubated under conditions similar to those in Figure 1, emphasizing the specificity of the reaction for the *second* G from **1**.

Detection of a New Signal in the EPR Spectrum of Vanadium Nitrogenase from *Azotobacter vinelandii*

Brian J. Hales,*,† Anne E. True,‡ and Brian M. Hoffman*,‡

Department of Chemistry
Louisiana State University
Baton Rouge, Louisiana 70803
Department of Chemistry
Northwestern University
Evanston, Illinois 60201
Received August 22, 1988

It is now well established that *Azotobacter vinelandii*[1,2] and *Azotobacter chroococcum*[3,4] can express alternative nitrogen-fixing enzymes that contain vanadium instead of molybdenum. The paramagnetism of the vanadium-containing protein of this enzyme from *A. vinelandii*[5] (called Av1′)[6] previously has been investigated and shown to exhibit at least two distinct EPR signals. In this communication, we report the discovery of a third EPR signal in the spectrum of the vanadium enzyme, which is difficult to detect in the absorption mode but is easily observed in the dispersion mode at pumped helium temperatures.

The first-derivative absorption EPR spectrum of Av1′ recorded at 14 K and 10 mW clearly shows two distinctly different signals, Figure 1, part A. The first signal (S1) has axial symmetry at $g \sim 2$ and most likely is associated with the $S = {}^1/_2$ spin system of a reduced Fe–S cluster. The second signal (S2), centered at $g = 5.5$, represents the low-field inflections of the ground-state and first-excited-state transitions of the two Kramers doublets of an $S = {}^3/_2$ spin system and has been tentatively assigned to a protein-bound VFe cofactor, analogous to the spin system of the MoFe cofactor of the conventional nitrogenase enzyme. It has been noted[5] that as the sample temperature is lowered and the incident microwave power increased (Figure 1, parts B and C), the recorded spectrum changes in several ways. Firstly, S2 changes in shape with temperature because it is composed of inflections from both the ground state and the first excited state,

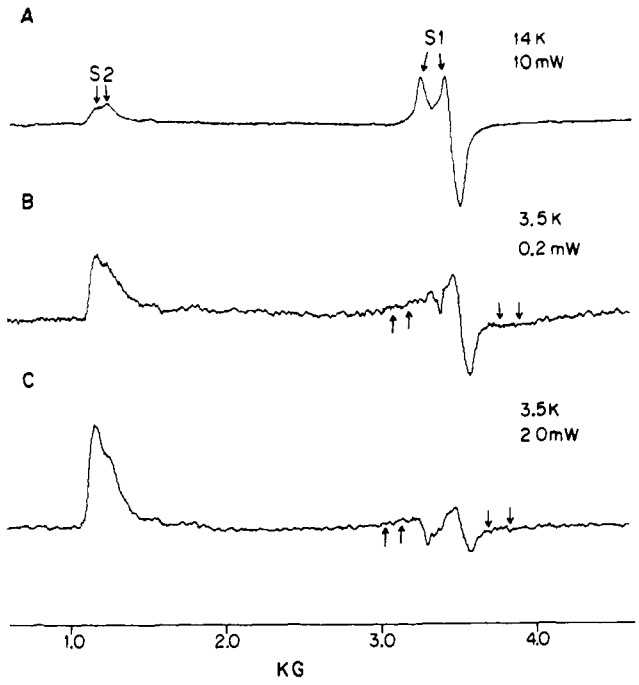

Figure 1. First-derivative absorption spectra of nitrogenase VFe protein (25 mM Tris buffer, pH 7.4, containing 0.25 M NaCl and 2 mM dithionite) from *Azotobacter vinelandii* showing the effects of varying temperature and incident microwave power on signals S1 and S2. Conditions: protein concentration, 25 mg mL^{-1}; sp act., 200 nmol of C_2H_2 reduced min^{-1} (mg of protein)$^{-1}$; modulation amplitude, 20 G; 100-kHz field modulation; frequency, 9.32 GHz; temperature and microwave power as indicated. Arrows in B and C point to the weak change in the baseline due to the center inflection of S3. This inflection occurs about $g \sim 2$ and, because it is the only portion of the S3 absorption with a major derivative amplitude change, is observable in this presentation under the conditions of high power and low temperature where S1 is saturated and S3 is enhanced.

and the Boltzmann populations change. The dependency of amplitude on power shows that this signal is difficult to saturate even at He temperatures. Secondly, S1, which saturates very easily at these temperatures, decreases dramatically with increasing power. In addition, at 3.5 K and high microwave power, a weak broad inflection of unknown origin also is observed[5] (see arrows in Figure 1, parts B and C) in the derivative absorption spectrum in the $g = 2$ region.

At even lower temperatures ($T \sim 2$ K) the EPR signals of most metalloproteins saturate readily and the electron spin–lattice relaxation time (T_1) and the field modulation frequency ($\omega_m = 2\pi\nu_m = 2\pi \times 10^5\ s^{-1}$) commonly obey the condition $\omega_m > 1/T_1$. Under this condition, when the saturated EPR signal of a frozen-solution sample is phase-sensitive detected with a spectrometer tuned to the dispersion mode, the response approximates to the undifferentiated absorption envelope.[7] Detection of EPR signals by this passage technique has the advantage that it enhances the detection of very broad and poorly resolved spectra and discriminates among overlapping signals that saturate at different levels of incident microwave power.

Figure 2 presents the passage signals of the VFe protein obtained as described above. With 0.02 mW of incident power, an absorption-like axial signal centered at $g \sim 2$, with width of ~250 G, dominates the EPR response; this corresponds to S1 in Figure 1. S2 is also weakly visible in this spectrum at ~1100 G.

A 10-fold increase of power to 0.2 mW has no substantial effect on the already-saturated S1 resonance but begins to bring the S2 signal into passage, as can be seen in the region with $H \geq 1100$ G. However, the major effect of the power increase is to disclose a new signal (S3), whose broad, structureless absorption envelope

† Louisiana State University.
‡ Northwestern University.

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