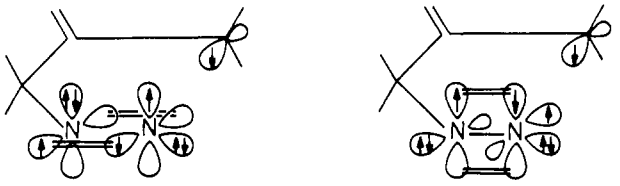
in trace amount (ca. 1%) the hydrazone **5** instead of the desired azo compound **1b**.[9] The minor product in the direct photolysis of azo compound **1a** was identical with hydrazone **5** by comparison with the authentic material by employing capillary GC/MS and GC/FTIR spectra and retention times on several capillary GC columns.

These results are mechanistically rationalized in eq 4. The most expedient way to explain the formation of hydrazone **5** is to accept the sequence **1a** ⇌ **2a** ⇌ **2b** ⇌ **1b** → **5**, involving first rupture of one C–N bond to afford the diazenyl diradical **1a**, which by C–C bond rotation leads to the rotameric diazenyl diradical **2b**, and final C–N bond reclosure yields the isomerized azo compound **1b**. The latter readily tautomerizes to the hydrazone **5**. Of course, the predominant competing reaction channel of the rotameric diazenyl diradicals **2a,b** is denitrogenation to afford the corresponding trimethylenemethane diradicals **3a,b**. Their subsequent fate is cyclization into the methylenecyclopropanes **4a,b**, respectively. The mechanistic intricacies of the latter process have recently been examined by means of deuterium labeling.[10]

What at first glance appears to be surprising about pyrazoline **1a** is the fact that its photolysis leads to isomerized azoalkane **1b** (eq 1), while its thermolysis does not,[8] although ample evidence for the involvement of diazenyl diradicals in the thermolysis of azo compounds has been documented.[11] A contrary case concerns the bicyclic azoalkanes of eq 2, in which the thermolysis led to isomerization but photolysis did not.[2] Even if structurally the "same" diradicals are involved in thermal and photochemical reactions, such species need not be identical. For example, they can differ in their vibrational excitation, i.e., "hot" vs. thermally equilibrated species,[12] in their spin states, i.e., singlet vs. triplet,[13] or in their electronic configuration,[14] i.e., σ- vs. π-type. Numerous examples exist which exemplify that the chemical fate of such molecular entities depends on such factors.[11] As we have postulated previously[7] on the basis of theoretical work,[6] photolysis (n,π^*-excitation) of azoalkanes should afford initially the π-type and thermolysis the σ-type diazenyl diradicals π-**2** and σ-**2**, respectively. While the latter is expected to readily denitrogenate



π - 2 σ - 2

to give the trimethylenemethane **3** and ground-state molecular nitrogen, the former would be obliged to generate **3** and n,π^*-excited molecular nitrogen. Thus, the photochemically produced diazenyl diradical π-**2** is expected to be longer lived, since nitrogen loss requires a greater activation energy and consequently formation of the isomerized azoalkane **1b** is more probable.[15]

It remains to be seen whether the intervention of diazenyl diradicals in the direct photolysis (n,π^*-excitation) of azo compounds is a general phenomenon, as seems to be implied in the theoretical study of the parent diazene.[6] It is our contention that diazenyl diradicals are more abundantly involved as intermediates than was previously implicated in the photolysis of cyclic azo compounds.

Acknowledgment. We thank the Deutsche Forschunggemeinschaft and the Fonds der Chemischen Industrie for generous financial support of this work.

Registry No. 1a, 55790-78-8; **1b**, 106251-08-5; **4a**, 54376-39-5; **4b**, 1121-36-4; **5**, 106251-09-6; diazomethane, 334-88-3; tetramethylallene, 1000-87-9.

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Nonenzymatic Sequence-Specific Cleavage of Single-Stranded DNA to Nucleotide Resolution. DNA Methyl Thioether Probes

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Received September 2, 1986

We report the sequence-specific cleavage of single-stranded DNA to nucleotide resolution using chemically activated oligodeoxynucleotide methyl thioether hybridization probes (DNA-MT). 5-[3-[[3-(Methylthio)propionyl]amino]-*trans*-1-propenyl]deoxyuridine 5′-triphosphate (MT-dUTP) was enzymatically incorporated into an oligonucleotide duplex by using the Klenow fragment of DNA polymerase. Activation with CNBr at 25 °C, pH 5.5, followed by treatment with piperidine produced cleavage on the complementary strand at a single guanine (G) residue to the 5′-side of the modified base.

Oligonucleotide probes capable of producing cleavage at a complementary strand have been reported, based on modification of nucleic acid strands with bifunctional reagents.[1] Using (2-chloroethyl)amine derivatives attached to the 5′-terminal phosphate of an oligonucleotide, Vlassov et al. observed alkylation and cleavage of three adjacent G residues on a single-stranded DNA target.[2] A second synthetic approach has been developed involving incorporation of a modified base into an oligonucleotide by chemical methods which allows the convenience of automated synthesis and affords control over the placement of the reactive group in the oligonucleotide strand. One example of this is DNA–EDTA·Fe(II) probes which oxidatively cleave several nucleotides on the complementary strand.[4]

The work described here involves enzymatic incorporation of a modified deoxyuridine triphosphate into a specific site on an oligonucleotide. To avoid the problem of premature inactivation and/or autocleavage of the modified DNA probe and to control the timing of the complementary strand cleavage, the cleaving function is masked. On the basis of the known sequence-specific cleavage of peptides with cyanogen bromide (CNBr) which results from the selective conversion of methionine to a highly reactive sulfonium species,[6] methyl thioether covalently attached to a DNA hybridization probe could be considered a latent alkylating moiety that can be selectively activated by CNBr under mild conditions.

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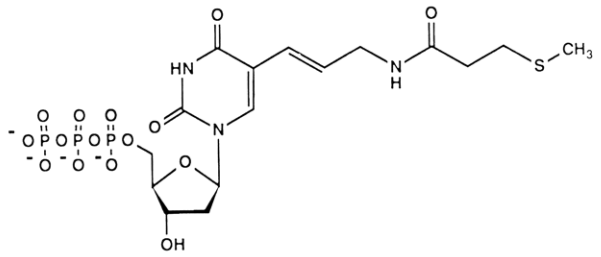

Figure 1. Structure of MT-dUTP.

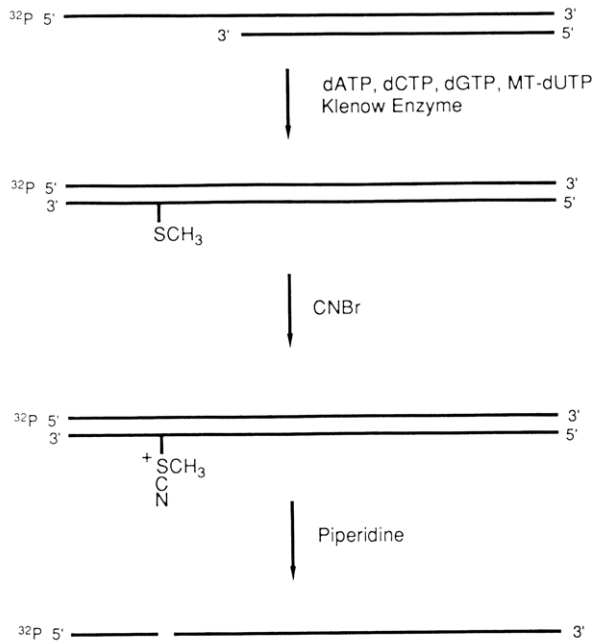

Figure 2. Scheme for enzymatic incorporation of MT-dUTP and CNBr activation of DNA methyl thioether probe (DNA-MT).

5-[3-[3-(Methylthio)propionyl]amino]-*trans*-1-propenyl]-deoxyuridine 5′-triphosphate (MT-dUTP, Figure 1) was synthesized by treatment of 5-[3-amino-*trans*-1-propenyl)deoxyuridine 5′-triphosphate with the *N*-hydroxysuccinimide ester of 3-methylthiopropionic acid (1:1 DMF/0.1 N sodium borate, 25 °C, pH 8.5).[7] MT-dUTP was purified by ion-exchange HPLC.[8,9] An oligonucleotide of sequence 5′-CTGTCTGGGGAGTCTCAGCAGTAGTCGTCATCAG-3′ was labeled with ^{32}P at the 5′-end by using T4 polynucleotide kinase and hybridized with an equimolar amount of the shorter complementary oligonucleotide, 5′-CTGATGACGAC-TACTGCTGA-3′. Using the Klenow fragment of DNA polymerase in the presence of dATP, dGTP, dCTP, and MT-dUTP, a single MT-dUTP was enzymatically incorporated to afford a fully double-stranded oligonucleotide (Figure 2).[10]

Assuming the DNA duplex forms the B structure, the electrophilic sulfonium species, generated by the reaction of the methyl thioether and CNBr, would be in the major groove proximal to the nucleophilic N-7 atoms on the neighboring G bases. To test the range of reactivity of the sulfonium species, four G residues were located on the ^{32}P-labeled complementary strand to the 5′-side of the adenine opposite the MT-dU base. A workup procedure utilizing piperidine (90 °C) should then cleave the phosphodiester backbone of the DNA at the site of any G alkylated at N-7.[11]

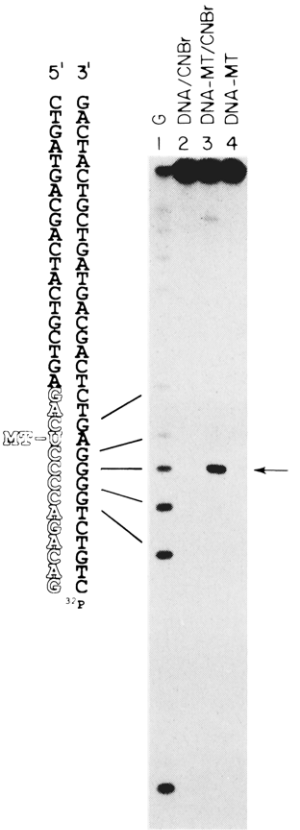

Figure 3. (Right) Autoradiogram of the 20% denaturing polyacrylamide gel. Lane 1: Maxam-Gilbert chemical sequencing G reaction.[11] Lane 2: Control of ^{32}P duplex labeled at the 5′-end with ^{32}P containing no modified uridine (dTTP was used in the Klenow reaction, instead of MT-dUTP), 1 μg of sonicated calf thymus DNA, 25 mM NaOAc, pH 5.5, 10 mM NaCl, and 20 mM CNBr. Lane 3: ^{32}P end-labeled duplex containing one enzymatically incorporated MT-dU, 1 μg of sonicated calf thymus DNA, 25 mM NaOAc, pH 5.5, 10 mM NaCl, and 20 mM CNBr. Lane 4: Control of ^{32}P end-labeled duplex containing one enzymatically incorporated MT-dU, 1 μg of sonicated calf thymus DNA, 25 mM NaOAc, pH 5.5, and 10 mM NaCl (*no* CNBr). All reactions were 10-μL total volume and were incubated at 25 °C for 5 h. In each workup 2 μL of neat piperidine was added and the reactions were heated at 90 °C for 20 min. The samples were frozen, lyophilized, and dissolved in formamide loading buffer. 0.2 mR/h was loaded onto each lane of the gel. (Left) The histogram shows the base positions filled in by Klenow enzyme (outlined bases) as well as the position of MT-dU in relation to the observed guanine (G) cleavage site (shown by arrow).

The purified duplex containing the DNA-MT probe was allowed to react with CNBr (20 mM, 5 h, 25 °C, pH 5.5) followed by workup with piperidine (16%, 20 min, 90 °C). The cleavage products of the ^{32}P-labeled strand were separated by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography. Cleavage was produced on the complementary strand at a single G located two base pairs to the 5′-side of the MT-U base (Figure 3, lane 3). Neighboring G bases were cleaved at least by a factor of 7 less. The efficiency of complementary strand cleavage is 11% based on uncleaved oligonucleotide.

The electrophoretic mobility of the fragments produced indicate that the cleavage reaction resulted in the production of 3′-phosphate ends consistent with an alkylation–depurination mechanism.[11] Although the mechanistic details of the cleavage reaction are not yet known, from CPK model building the base position cleaved is the one nearest the alkyl sulfonium if one assumes an S_N2 geometry for transalkylation at N-7 of G. Presumably the strict geometric requirements in the transition state for an intramolecular nucleophilic substitution reaction[12] are responsible

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(9) NMR and UV spectra confirm the structure of MT-dUTP. Synthetic details will be published elsewhere.

(10) Controls demonstrate that in the absence of MT-dUTP, a nucleotide triphosphate was not incorporated opposite the adenine in the overhang region of the partial duplex.

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for the observed specificity.[13] As a control, a similar oligonucleotide duplex was prepared which contained thymidine and no modified uridine. When this duplex was treated with CNBr followed by piperidine workup, no specific cleavage was observed (Figure 3, lane 2).[14] In addition, DNA-MT probe in the absence of CNBr does not cleave DNA (Figure 3, lane 4).

The sequence-specific cleavage of DNA is useful in many techniques in molecular biology including DNA sequence determination, chromosome mapping, gene isolation, and recombinant DNA manipulations. This work has demonstrated an enzymatic route to synthesize DNA methyl thioether hybridization probes, chemical activation of methyl thioether with CNBr to initiate complementary DNA strand cleavage, and nonenzymatic sequence-specific cleavage of single-stranded DNA at guanine to nucleotide resolution.[15]

Acknowledgment. We are grateful to the National Institutes of Health (GM-35724) for support of this research and for a National Research Service Award (T32GMO7616) to B.L.I. from the National Institute of General Medical Sciences.

(13) This experimental result defines an important distance in nucleic acid duplexes (assuming that the 5-substituent of MT-U is fully extended) i.e., the cross-helix distance required for S_N2 reactions. In this regard, MT-dUTP analogues with one additional or deleted methylene unit between the amide carbonyl and methyl thioether group were also synthesized. When incorporated into DNA-MT hybridization probes, the shorter analogue showed very little cleavage and the longer analogue showed specificity similar to MT-dUTP but with lower efficiency.

(14) No cleavage at that position was observed when adenine was substituted in the complementary strand at the unique position of G cleavage.

(15) Complete characterization of the DNA cleavage products will be reported in due course.

Photochemistry of (5,10,15,20-Tetraphenylporphyrinato)iron(III) Halide Complexes, Fe(TPP)(X)

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Received September 8, 1986

The photochemistry of metalloporphyrins and related macrocycles is of intense current interest.[1] Nonetheless, the photochemistry of ferric porphyrins and heme proteins remains largely unexplored and not well understood.[2] The ability of Fe(III) porphyrins to act as catalysts for hydrocarbon oxidations with various oxidants[3,4] suggested to us their possible use as photocatalysts. In this paper, we assign a near-ultraviolet absorption in $Fe^{III}(TPP)(X)$ (where X = F, Cl, Br, I, N_3; TPP =

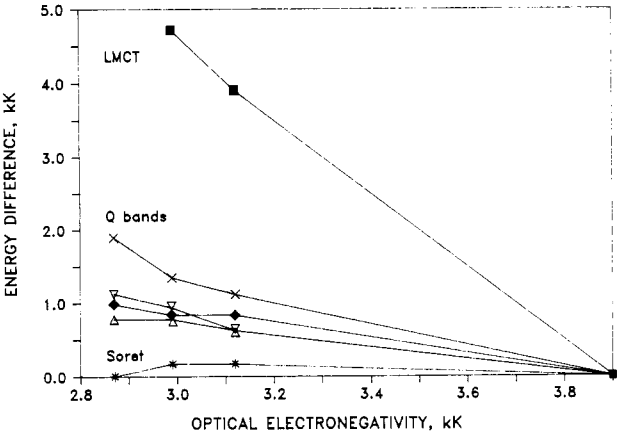

Figure 1. Spectral changes for Fe(TPP)(X) as a function of the optical electronegativity of X. From left to right, X is I, Br, Cl, and F; energy differences were calculated against Fe(TPP)(F). The LMCT band is in the region 350–400 nm; the Soret band is around 420 nm; the Q bands are in the region 450–700 nm.

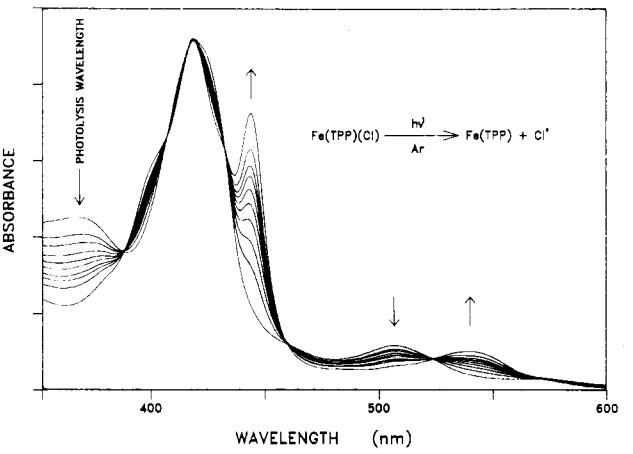

Figure 2. Photoreduction of $Fe^{III}(TPP)(Cl)$ to $Fe^{II}(TPP)$ in cumene under Ar upon irradiation of the near-ultraviolet LMCT band. Similar spectra were observed in other solvents and with other anionic ligands.

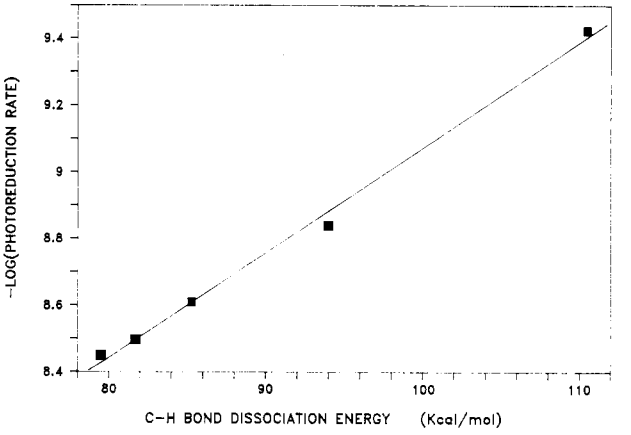

Figure 3. Linear free energy relationship between the rate of photoreduction of Fe(TPP)(Cl) and solvent bond dissociation energy. From left to right, the solvents are cumene, ethylbenzene, toluene, cyclohexane, and benzene. The bond dissociation energies plotted are for the solvent's most easily abstracted hydrogen; photoreduction rates have been normalized per abstractable hydrogen. The solid line is a linear regression fit.

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