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]

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(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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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 =

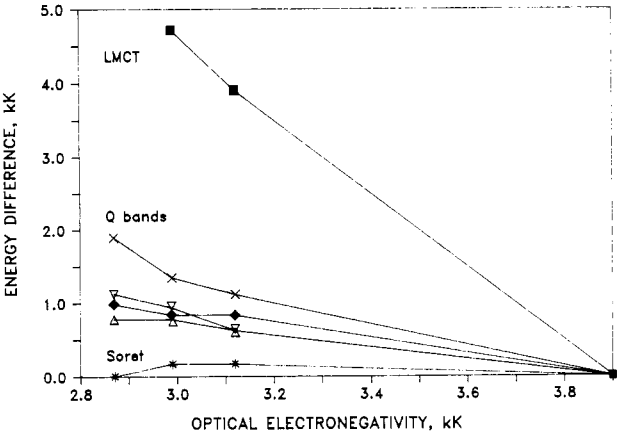

Figure 1. Spectral changes for Fe(TPP)(X) as a function of the optical electronegativity of X. From left to right, X is I, Br, Cl, and F; energy differences were calculated against Fe(TPP)(F). The LMCT band is in the region 350–400 nm; the Soret band is around 420 nm; the Q bands are in the region 450–700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

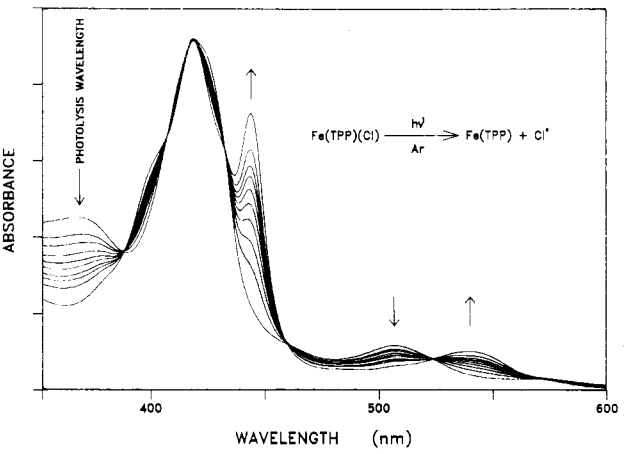

Figure 2. Photoreduction of $Fe^{III}(TPP)(Cl)$ to $Fe^{II}(TPP)$ in cumene under Ar upon irradiation of the near-ultraviolet LMCT band. Similar spectra were observed in other solvents and with other anionic ligands.

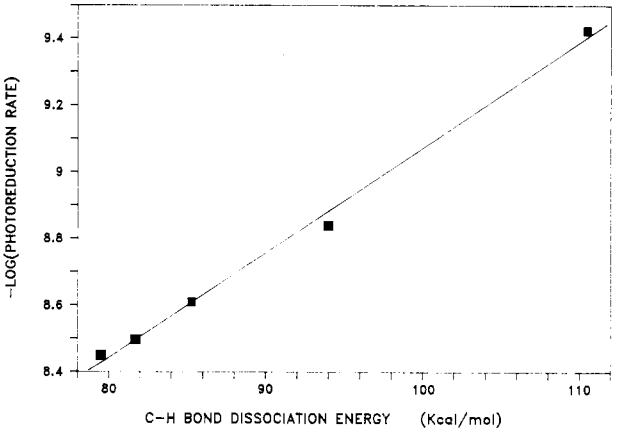

Figure 3. Linear free energy relationship between the rate of photoreduction of Fe(TPP)(Cl) and solvent bond dissociation energy. From left to right, the solvents are cumene, ethylbenzene, toluene, cyclohexane, and benzene. The bond dissociation energies plotted are for the solvent's most easily abstracted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

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5,10,15,20-tetraphenylporphyrinate(-2)) as a halide ligand-to-metal charge-transfer (LMCT) transition. Irradiation into this band leads to rapid photoreduction of the iron atom and dissociation of X•. In the presence of O_2, photoinitiation of hydrocarbon oxidation occurs.

Most of the features in the optical absorption spectrum of Fe(porph)(X) complexes are due to porphyrin-localized $\pi-\pi^*$ transitions.[5] A strong band to higher energy than the Soret band has not been analyzed in the literature. The influence of axial ligation on the energy of this transition permits the unambiguous assignment of this band as a halogen-to-metal charge transfer. In comparison to the Soret or Q-bands, which are $\pi-\pi^*$ transitions and therefore virtually unaffected by the choice of axial anion, this LMCT band shifts strongly to lower energy as the ligand's electronegativity decreases, as shown in Figure 1.

Irradiation into this CT band, and *only* into this band, causes the clean photoreduction of the iron atom in the absence of O_2 (as shown in Figure 2). The rate of Fe^{II}(TPP) production has a strong solvent dependence: cumene > ethylbenzene > toluene > cyclohexane. As shown in Figure 3, an excellent linear free energy relationship exists between the photoreduction rates and the solvents' bond dissociation energies. This is consistent with the abstraction of hydrogen atoms from the solvent as a key step in the overall photoreduction of the iron porphyrin. For Fe(TPP)(Cl) in a 1 M solution of cumene in benzene, the photoreduction quantum yield over 362 ± 11 nm is 5.1×10^{-4}. Laser flash photolysis (at 355 nm) of Fe(TPP)(I) indicates that Fe^{II}(TPP) is formed within the lifetime of the laser pulse (10-ns fwhm). In benzene and in the absence of O_2, this transient does not decay on the nanosecond timescale and only partially returns after tens of microseconds. Our data are consistent with the following mechanism:

$$\text{Fe(TPP)(X)} \xrightarrow[\text{LMCT}]{h\nu} \text{Fe(TPP)} + \text{Cl}^\bullet \quad (1)$$

$$\text{Fe(TPP)} + \text{Cl}^\bullet \rightarrow \text{Fe(TPP)(Cl)} \quad (2)$$

$$\text{Cl}^\bullet + \text{RH} \rightarrow \text{HCl} + \text{R}^\bullet \quad (3)$$

$$\text{R}^\bullet \rightarrow \text{products} \quad (4)$$

Radicals are known to react with Fe(II) porphyrins under acidic, aqueous conditions to give Fe(III) and alkanes.[6] Under our very different conditions, however, we find no evidence for such reoxidation, presumably due to the effectiveness of the secondary reactions of R• (e.g., R-R formation).

This photoreduction mechanism suggests that photocatalytic hydrocarbon hydroxylation with O_2 should be possible via a peroxyl radical chain autooxidation.[7] In fact, upon photolysis of Fe(TPP)(X) in the presence of both substrate and O_2, photoinitiation of hydrocarbon oxidation is observed. In a typical experiment, a benzene solution which was 1 M in substrate and 1 mM in Fe(TPP)(Cl) was irradiated with a Xe arc lamp filtered at 362 ± 6 nm; aliquots were removed periodically and analyzed by capillary GC and GC/MS. For cyclohexene, the expected allylic oxidation products are found (cyclohexen-3-ol (23%) and cyclohexen-3-one (77%)) with a quantum yield of 0.26 and with 150 equiv of products produced per equiv of porphyrin consumed. For cumene, the usual autoxidation products are formed (cumyl alcohol (76%) and acetophenone (24%)), with a quantum yield of 0.30 and with 160 equiv of products produced per equiv of porphyrin consumed. Other substrates with lower oxidizability ratios[7] (e.g., toluene) are not hydroxylated to a significant degree. The eventual fate of the iron porphyrin is observed to be principally $(FeTPP)_2O$, derived from the autoxidation of Fe(TPP), which may be reconverted to Fe(TPP)(Cl) upon addition of HCl.[8]

These results demonstrate that the photochemistry of even simple Fe(III) porphyrins is quite rich and allows a new entry into the oxidation of hydrocarbons with O_2. Further work on the photochemistry of metalloporphyrin complexes with oxoanions is under way.[9]

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Interconversion of Conformation of Apomyoglobin Adsorbed on Hydrophobic Silica Gel

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We wish to report what is, to our knowledge, the first direct observation of multiple conformations and of interconversion between them, for a protein adsorbed to a solid surface. The behavior of proteins adsorbed at solid-liquid interfaces is an area of considerable interest.[1,2] A key concern is the degree of reorganization in the three-dimensional protein structure, if any, that accompanies adsorption. The results from the few studies to date show that changes in protein conformation upon adsorption may range from undetectable to substantial,[3,4] depending on the protein and substrate. A promising technique is intrinsic fluorescence spectroscopy,[5] recently employed to probe changes in fibronectin conformation upon adsorption to hydrophobic silica.[6] We report here on the direct observation of the intrinsic fluorescence characteristics of apomyoglobin in the adsorbed state on a hydrophobic, microparticulate silica gel—Zorbax 100.[7] Our data suggest that the degree of reorganization of the protein in the adsorbed state is dependent on the pH of the contact buffer. At acid pH, the protein is more unfolded and interacts to a greater extent with the surface than at neutral pH and partial reversibility between the two surface conformers is observed.

Sperm whale apomyoglobin was prepared by the methyl ethyl ketone method.[8] Protein solutions were buffered in 20 mM phosphate. The quartz column flow cell[9] was packed with fresh

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