Table I. Comparison of Calculated and Experimental Isotropic Hyperfine Couplings for $1^{\bullet+}$

interatomic distances[a] (pm)				nuclei	AM1[b] spin densities ρ_s	AM1[b] spin densities ρ_s[d]	INDO[c] spin densities ρ_s	calcd hfcs (G) from INDO spin densities	expt. hfcs (G)
C(1)-N(2)	155.2	$C(1)-H_{br}$	112.0	$2^{14}N$	0.0474	0.0517	0.0512	19.4,[e] 28.1,[f] 33.1[g]	31.0
N(2)-N(3)	117.3	$C(5)-H_{syn}$	112.2	$2^{1}H_{br}$	-0.0107	-0.0033	-0.0065	-3.5,[h] -3.3[i]	(3.6)[j]
C(1)-C(6)	154.2	$C(5)-H_{anti}$	112.5	$4^{1}H_{syn}$	0.0010	0.0010	0.0025	1.4,[h] 1.3[i]	
C(5)-C(6)	152.6			$4^{1}H_{anti}$	0.0243	0.0177	0.0267	14.4,[h] 13.5[i]	15.5[k]

[a] Optimized geometry by AM1 method corresponding to a ΔH_f for $1^{\bullet+}$ of 241.985 kcal/mol. The CNN angle is 117.3 deg. [b] Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. [c] Pople, J. A.; Beveridge, D. L.; Dobosch, P. A. *J. Chem. Phys.* **1967**, *47*, 2026. [d] After spin annihilation. [e] Using the INDO proportionality constant of 379.4 G. [f] Using the calculated atomic value of 550 G (Morton, J. R.; Rowlands, J. R.; Whiffen, D. H. *National Physical Laboratory Bulletin*; no. BPR 13, 1962). [g] Using the calculated atomic value of 646 G (Morton, J. R.; Preston, K. F. *J. Magn. Reson.* **1978**, *30*, 577). [h] Using the INDO parameter of 540 G. [i] Using the atomic value for hydrogen of 506.7 G. [j] Measured from hf substructure of parallel features in the anisotropic spectrum recorded in $CFCl_3$ at 130 K. [k] ENDOR measurements[9] give 15.09 G.

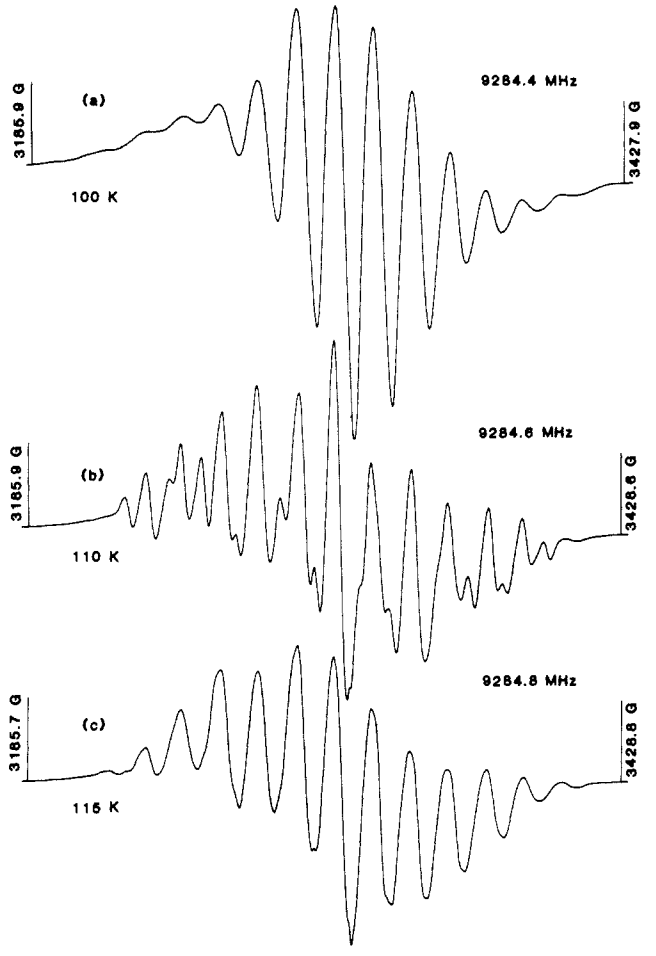
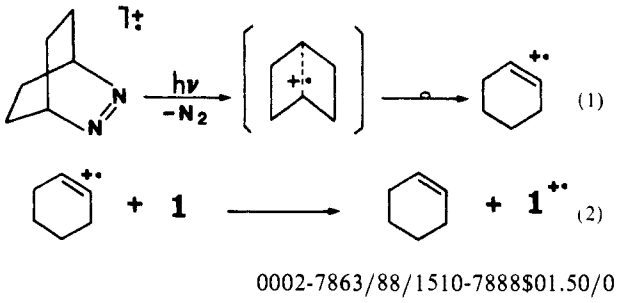


Figure 2. ESR spectrum of a γ-irradiated 1 mol% solution of 2,3-diazabicyclo[2.2.2]oct-2-ene in $CF_2ClCFCl_2$ (dose, 0.3 Mrad) recorded consecutively (a) at 100 K, (b) at 110 K after photobleaching at 100 K with blue light ($\lambda < 400$ nm; glass filter C. S. no. 7-54) from a 450-W xenon lamp, and (c) at 115 K. Spectra (a), (b), and (c) are assigned to $1^{\bullet+}$, cyclohexene$^{\bullet+}$, and $1^{\bullet+}$ respectively. The resolution of the inner lines of the cyclohexene$^{\bullet+}$ spectrum depends on both the freon matrix and the temperature.

the spectrum of $1^{\bullet+}$ reappeared on warming the $CF_2ClCFCl_2$ matrix from 110 to 115 K (Figure 2, (b) and (c)). This thermal transformation does not occur in $CFCl_3$ and can be attributed to the bimolecular electron-transfer reaction 2[16] which becomes possible in the mobile $CF_2ClCFCl_2$ matrix.[17] Thus, reactions 1 and 2 constitute a photochemically assisted chain reaction for the conversion of **1** to cyclohexene via their radical cations, the loss of nitrogen in the photofragmentation of $1^{\bullet+}$ resulting in a more powerful oxidant which regenerates $1^{\bullet+}$.



Acknowledgment. We are indebted to Sheng Dai for his help with the computations. Professor F. Gerson (University of Basel) kindly informed us that proton ENDOR measurements agree with the interpretation of the ESR spectrum of $1^{\bullet+}$ given here and in the preprint of our communication, and we also thank Professor P. H. Rieger (Brown University) for his interest in the work. This research was supported at the University of Tennessee by the Division of Chemical Sciences, U.S. Department of Energy (Grant DE-FG05-88ER13852), and at the University of Wisconsin by the National Science Foundation (Grant CHE-8415077).

(16) The exothermicity of reaction 2 is estimated to be 0.62 eV from the difference in vertical ionization potentials of cyclohexene (8.94 eV)[16a] and **1** (8.32 eV).[6] (a) Kimura, K.; Katsumata, S.; Achiba, Y.; Yamazaki, T. *Handbook of He I Photoelectron Spectra of Fundamental Organic Molecules*; Japanese Scientific Societies Press: Tokyo, 1981; p 67.

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Photosensitized Cleavage of a Thymine Dimer by an Antibody

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The development of monoclonal antibody technology has provided ready access to homogeneous, high affinity ligand binding sites which recognize a large number of structurally diverse molecules.[3] Consequently, the development of strategies for the introduction of catalytic activity into antibodies should allow the design of biological catalysts with a wide range of specificities. One such strategy involves the generation of antibodies whose binding sites are complementary to the rate-limiting transition state of the reaction of interest. For example, antibodies elicited to transition-state analogues for acyl transfer and pericyclic reactions were found to accelerate the corresponding reactions 10^4-10^6-fold.[4-10] Alternatively, it should be possible to obtain

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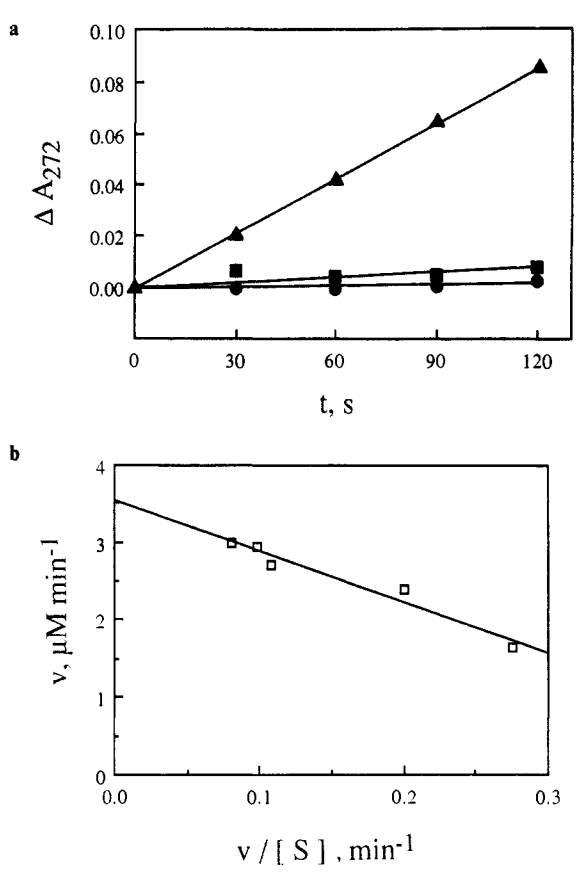


Figure 1. (a) Increase in absorbance at 272 nm (thymine monomer) as a function of irradiation time for reaction mixtures containing 25 μM dimer **1** and 3 μM antibody 15F1-3B1 (▲), 3 μM MOPC 315 (dinitrophenyl specific) (●), or no antibody (■). (b) Eadie–Hofstee plot for the antibody-sensitized cleavage of dimer **1**.

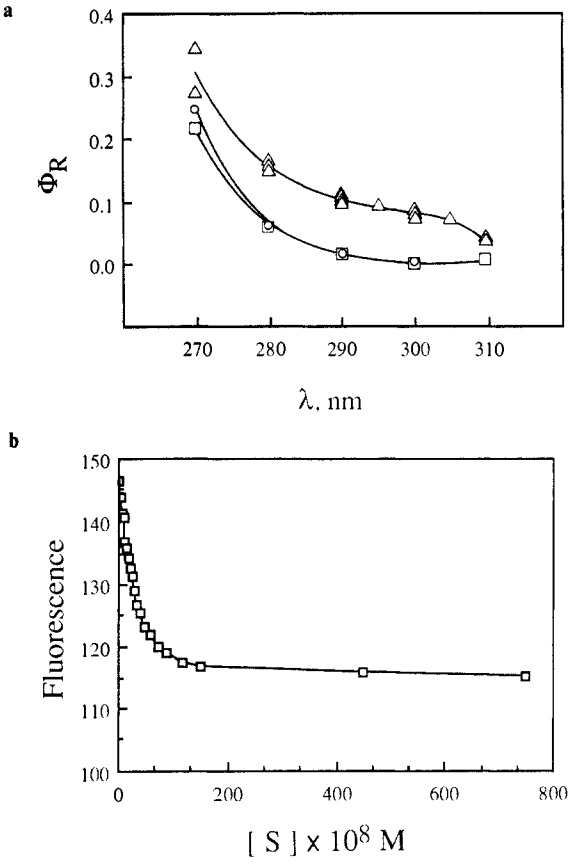
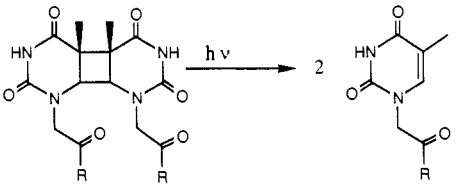


Figure 2. (a) Action spectrum showing the dependence of the quantum yield for monomer formation on the wavelength of irradiation. The reaction mixtures contained 264 μM hapten **2** and 3 μM dimer-specific antibody 15F1-3B1 (△) or one of the nonspecific antibodies MOPC 315 (□) and MOPC 167 (○). (b) Fluorescence (arbitrary units; excitation: 280 nm, emission: 348 nm) of the antibody 15F1-3B1 as a function of added ligand **2**. Fluorescence quenching experiments were performed with 0.2 μM antibody at 18 °C in the same buffer used for the photolysis experiments.[22]

an antibody with a catalytic amino acid side chain in the binding site by design of an antigen with structural features complementary to that side chain. We report here application of this latter strategy to the generation of antibodies which catalyze the photocleavage of thymine cyclobutane dimers, the predominant DNA photolesion produced by UV light.[11]

Organisms have evolved a number of mechanisms for the repair of pyrimidine dimers, including the photoreactivating enzyme DNA photolyase which cleaves thymidine dimers upon irradiation with visible light ($\lambda > 300$ nm).[12,13] Although the mechanism of enzymatic repair remains unclear, model studies suggest that photosensitizers such as indoles, quinones, or flavins can reversibly transfer an electron to or accept an electron from the dimer, resulting in facile cleavage of the intermediate thymine dimer radical.[14-16] These results suggest that an antibody combining

Scheme I



1: R = OH

2: R = $NHCH_2COOH$

site specific for a thymine dimer and containing an appropriately positioned sensitizer should act as a photoreactivating enzyme. It seemed reasonable to us that antibodies generated against the polarized π system of a pyrimidine dimer might contain a complementary tryptophan residue in the combining site, much as antibodies generated against positively charged haptens contain complementary aspartate and glutamate residues.[17]

In order to test this strategy, antibodies were generated against *cis,syn*-thymine dimer **2** (Scheme I). The stereochemistry of the photocyclization reaction leading to **2** was controlled via dimerization of the *N*-1-carboxymethyl derivative, as the ethylene glycol diester.[18,21] Dimer **2** was coupled via its *N*-hydroxysuccinimide

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ester to the carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) and exhaustively dialyzed against 150 mM NaCl, 10 mM phosphate buffer, pH 7.5. Epitope densities ranged between 5 and 15. Balb/c mice were immunized with the KLH conjugate of **2**, and antibodies were generated by standard protocols.[6] IgG was purified from ascites fluid by affinity chromatography on protein A-coupled Sepharose 4B and judged homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.[22]

Solutions of carboxymethylthymine dimer **1** were irradiated with 300 nm light in the presence of antibody; dimer cleavage was assayed spectrophotometrically.[23] Five out of six antibodies (IgG) were found to sensitize the photocleavage. One of these, antibody 15F1-3B1 was studied further. High pressure liquid chromatography confirmed that the reaction product was thymine monomer. The absorption and fluorescence spectra of the antibody remained unchanged upon photolysis, indicating that photodegradation of the protein was negligible. The kinetics of the antibody-catalyzed reaction are consistent with the Michaelis-Menten rate expression (Figure 1):

$$\mathrm{Ig} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Ig{\cdot}S} \xrightarrow{k_{cat}} \mathrm{Ig} + \mathrm{P}$$

The kinetic constants k_{cat} and K_m are 1.2 min^{-1} and 6.5 μM, respectively ($k_{cat}/K_m = 1.8 \times 10^5$ M^{-1} min^{-1}). The k_{cat} value observed is comparable to the turnover number for *Escherichia coli* DNA photolyase of 3.4 min^{-1}.[24] The reaction is first order in light between incident intensities of 5×10^{-8} and 1.6×10^{-7} einsteins min^{-1}. Consequently, the k_{cat} obtained is not optimal; irradiation at higher flux should increase k_{cat} until light saturation occurs.[25] The first-order rate constant for unsensitized dimer cleavage is 5.5×10^{-3} min^{-1}. Hapten **2** is also readily cleaved by the antibody; however the K_m for this substrate is too low to be conveniently measured by direct spectrophotometric assay (<1 μM). The photocleavage of the corresponding *N,N'*-dimethyl substrate[18] is not sensitized by the antibody at substrate concentrations of up to 1.8 mM, which is consistent with the high specificity of antibody-ligand binding. In addition, the dinitrophenyl specific IgA MOPC 315, which contains a binding-site tryptophan, and the phosphorylcholine specific IgA MOPC 167 do not catalyze the photocleavage of thymine dimer under identical reaction conditions.

The wavelength dependence of the quantum yield of the antibody-sensitized photolysis of hapten **2** reveals a shoulder at 300 nm (Figure 2a). Antibody fluorescence is also quenched in the presence of thymine dimer (Figure 2b: It should be noted that the percentage of fluorescence quenched upon ligand binding does not reflect the total number of tryptophan residues in the protein due to environmental effects on Φ_F). These observations suggest that a combining site tryptophan is photosensitizing dimer cleavage. The quantum yield of the antibody-catalyzed reaction ($\Phi_{R,300}$) is 0.08. The antibody was oxidized with *N*-bromosuccinimide in 8 M urea, pH 4.5, revealing $\geq$10 tryptophan residues per Ig.[26] Assuming that each binding site contains only one tryptophan allows the calculation of an approximate quantum yield of $\geq$0.4 for photocleavage of bound dimer (based on formation of monomer). Pulse radiolysis experiments have shown that dimethyl and tetramethylthymine dimer radical anions decay to monomer with a frequency of 0.05.[27] Since the quantum yield of the antibody-sensitized reaction is significantly higher than 0.05, the antibody appears to partition the breakdown of the intermediate radical anion. Further experiments are being carried out to precisely define the mechanism of this antibody-catalyzed reaction.

This work represents the extension of antibody catalysis to a new class of reactions. Moreover, it should be possible to utilize antibody-hapten complementarity to generate antibodies with active-site amino acid residues which selectively catalyze a variety of other reactions, including hydrolyses, isomerizations, and eliminations.

Acknowledgment. We thank W. G. Dauben, G. Ganzer, J. E. Hearst, and H. Frei for assistance in carrying out the photochemical experiments and T. Begley for helpful discussions. Financial support was provided by the Office of Naval Research (ONR N0014-87-K0256) to P.G.S.

(18) Hapten **2** was synthesized by alkylation of thymine with chloroacetic acid in aqueous potassium hydroxide.[19] Carboxymethylthymine was treated with 1 equiv of *N,N'*-carbonyldiimidazole (CDI) in dimethylformamide (DMF) followed by addition of 0.5 equiv of ethylene glycol. Removal of solvent and trituration with water yielded the ethylene glycol diester. The diester was dissolved in minimal DMF and photolyzed in degassed 10% aqueous acetone (1 g/750 mL) through a Pyrex filter for 2 h with a 450-W Hanovia medium pressure Hg immersion lamp. Removal of solvent and trituration with water afforded the thymine dimer ethylene glycol diester as a single isomer (cis, syn). Basic hydrolysis and subsequent acidification to pH 2 gave crystalline carboxymethylthymine-*cis,syn*-cyclobutane dimer **1**. Activation with CDI in DMF followed by addition of glycine ethyl ester yielded the bis[ethyl glycinate] adduct after removal of solvent and trituration with water. Basic hydrolysis and removal of solvent gave hapten **2**. Dimethylcarboxymethylthymine-*cis,syn*-cyclobutane dimer was prepared by alkaline dimethyl sulfate treatment[20] of the carboxymethylthymine ethylene glycol diester photoproduct followed by adjustment of the pH to 11 for 1 h and subsequent acidification to pH 2 to induce crystallization.

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(23) Deoxygenated solutions of thymine dimer (5-300 μM) and antibody (3 μM) in 100 mM NaCl, 10 mM phosphate buffer, pH 7.5, were irradiated at 18-20 °C in a 1-mL quartz cuvette. All photolysis experiments were performed with a 1000-W high pressure Hg-Xe lamp equipped with a Photon Technologies International, Inc. Model Q5001 Quantacount electronic actinometer and a diffraction grating monochromator blazed at 500 nm. A bandpass of 3 nm was used for both kinetic and photon counting experiments. Kinetic experiments were performed at 300 nm with an incident flux of 1.26×10^{-7} einsteins min^{-1}. Monomer formation was assayed spectrophotometrically at fixed time intervals (λ_{max} = 272 nm, log ϵ = 3.90). Protein molarity was determined by absorbance at 280 nm by using $\epsilon^{0.1\%} = 1.37$ and a molecular weight of 150 kD for IgG.

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The Mechanism of Hydroperoxide O-O Bond Scission on Reaction of Hydroperoxides with Iron(III) Porphyrins

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An understanding of the mechanism of reaction of RCO_3H and alkyl-OOH compounds with iron(III) porphyrins is important in the understanding of the mechanisms of reactions of horseradish peroxidase, catalase, and cytochrome P-450 enzymes. Heterolytic O-O bond breaking is involved in the reactions of alkyl-OOH and RCO_3H species with (EDTA)(Fe^{III}) (in methanol) and (*meso*-tetraphenylporphinato)chromium(III) chloride ((TPP)Cr^{III}(Cl)) (in CH_2Cl_2). The log of the apparent second-order rate constants (k_{ly}) for reaction of both alkyl-OOH and RCO_3H species (YO-OH) when plotted vs the pK_a of alkyl-OH and RCO_2H (YOH) leaving groups falls on single lines with (EDTA)(Fe^{III})[1] and with ((TPP)Cr^{III}(Cl)).[2,7b] In marked contrast, a plot of log k_{ly} vs pK_a

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