Supplementary Material Available: A table of the UHF and RHF energies, dipole moments, and $\langle \hat{S}^2 \rangle$ values for the SCRF calculations (2 pages). Ordering information is given on any current masthead page.

Antibody-Catalyzed Redox Reaction†

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It is now well established that antibodies can attain enzyme-like attributes.[1] Several examples of acyl transfer reactions,[2] a pericyclic reaction,[3] and a β-elimination[4] have been subject to acceleration by antibodies. Other types of reactions, such as oxidation-reduction, are less obvious candidates for antibody catalysis for lack of a general strategy for defining mimics of an activated complex.

A common feature of oxidoreductases is the existence of multiple binding sites for substrates and cofactors. Therefore any attempt to use antibodies as redox catalysts should address the possibility for two or more ligands to simultaneously occupy the combining site. We recently described the multiligand binding properties of anti-fluorescyl antibodies.[5] Their affinity for fragments of the hapten is consistent with molecular recognition at adjacent subsites of the combining region. The recognition of various reducible dyes in proximity to a secondary binding site for carboxylates suggested the possibility for redox chemistry between ligands bound at the two subsites. Here we report the catalytic activity of antifluorescyl antibodies in the reduction of a dye substance, resazurin (**1**), by sulfite.

The benzoate fragment of fluorescein is rigidly aligned with the xanthenyl fragment through a carbon-carbon bond to the C(9) position. The carboxylate group is oriented above the plane of the tricyclic group and as appropriate for nucleophilic addition or electron transfer to the electrophilic atoms of a xanthene or phenoxazine moiety. Antibodies to a fluorescyl hapten may be capable of aligning two substrates in a chemically productive complex (Scheme I). The dye resazurin has been used extensively as a redox indicator,[6] and its analogy with 3,6-dihydroxyxanthenes makes it a good ligand for anti-fluorescyl antibodies.

Resazurin is reduced by sulfite (or bisulfite)[7] to produce resorufin (**2**) as determined by spectroscopic and chromatographic identification.[8] The reaction rate is first order in each substrate with a second-order rate constant at pH 5.8, $k_{uncat} = 0.104\ M^{-1}\ s^{-1}$. Linear dependence of log k_{uncat} on pH, increasing with de-

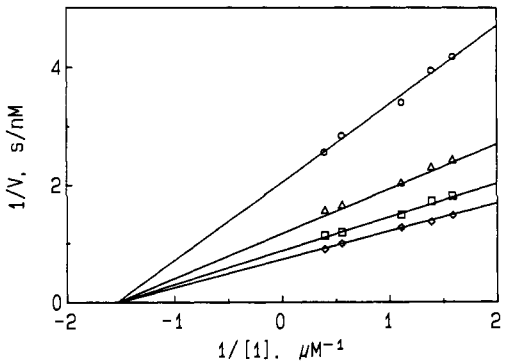


Figure 1. Lineweaver-Burk plot for reduction of **1** by 0.5 (○), 1.0 (△), 1.5 (□), and 2.0 (◇) mM sulfite catalyzed by monoclonal antibody (MAb) 66D2 at 1.4×10^{-7} M. Initial catalytic rates were determined by measuring the absorbance decrease at 605 nm ($\epsilon_{605} = 4.3 \times 10^4$ au $M^{-1}\ cm^{-1}$) at pH 5.8 and 25.0 ± 0.1 °C in 10 mM Bis-Tris buffer containing 80 μM EDTA, correcting for the uncatalyzed rate. Linear regression *y* intercepts and slopes from this plot were used in secondary plots to obtain the values of k_{cat}, K_m^S, and K_m^I. These values were used to draw the lines shown.

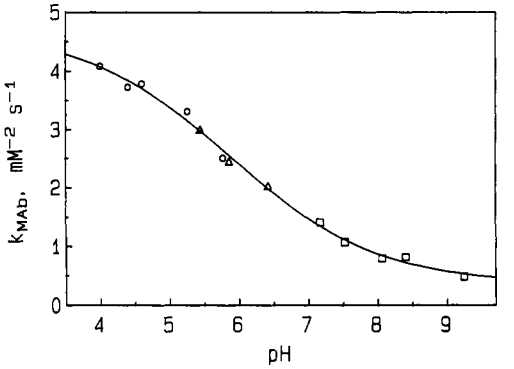
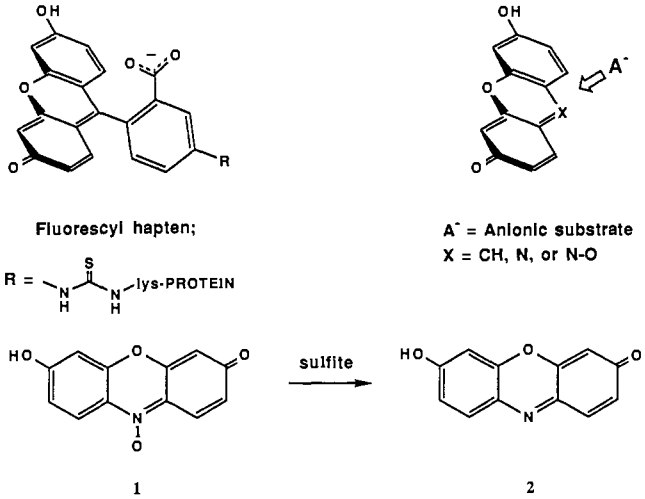


Figure 2. The pH dependence of 66D2-catalyzed reduction of **1** (2.6 μM) with sulfite (1 mM) in 2 mM pyridine (○), Bis-Tris (△), and Tris (□) buffers measured as described in Figure 1. First-order dependence in 66D2 allows calculation of k_{MAb} from plots of k_{obsd} vs [MAb] ($V_{obsd} = \{k_{uncat} + k_{MAb}[MAb]\}[sulfite][1]$).

Scheme I



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(1) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; p 288.

(2) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Science (Washington, D.C.)* **1986**, *234*, 1566. Pollack, S. J.; Jacobs, J. W.; Schultz, P. G. *Science (Washington, D.C.)* **1986**, *234*, 1570. Napper, A.; Benkovic, S. J.; Tramontano, A.; Lerner, R. A. *Science (Washington, D.C.)* **1987**, *237*, 1041. Jacobs, J. W.; Schultz, P. G.; Sugasawara, R.; Powell, M. *J. Am. Chem. Soc.* **1987**, *109*, 2174. Tramontano, A.; Ammann, A. A.; Lerner, R. A. *J. Am. Chem. Soc.* **1988**, *110*, 2282. Janda, K. D.; Lerner, R. A.; Tramontano, A. *J. Am. Chem. Soc.* **1988**, *110*, 4837. Janda, K. D.; Schloeder, D.; Benkovic, S. J.; Lerner, R. A. *Science (Washington, D.C.)* **1988**, *241*, 1188. Benkovic, S. J.; Napper, A. D.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5355. Iverson, B. L.; Lerner, R. A. *Science (Washington, D.C.)* **1988**, *243*, 1184. Pollack, S. J.; Hsiun, P.; Schultz, P. G. *J. Am. Chem. Soc.* **1989**, *111*, 5961.

(3) Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 4841. Hilvert, D.; Carpenter, S. H.; Nared, K. D.; Auditor, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4953.

(4) Shokat, K. M.; Leumann, C. J.; Sugasawara, R.; Schultz, P. G. *Nature (London)* **1989**, *338*, 269.

(5) Janjić, N.; Schloeder, D.; Tramontano, A. *J. Am. Chem. Soc.* **1989**, *111*, 6374.

(6) Twigg, R. S. *Nature (London)* **1945**, *155*, 401. DeBaun, R. M.; Kudzin, S. F.; Schubert, W. J. *Arch. Biochem.* **1950**, *26*, 375.

(7) With a pK_a of 6.9, HSO_3^- is the predominant ionization state in acidic pH.

(8) The reaction product has a UV-vis spectrum in alkaline pH (λ_{max} at 571 nm, shoulder at 535 nm) identical with that of resorufin. The product also coelutes with resorufin by TLC and reversed-phase HPLC (Vydac 201TP54 C-18 column; 1:1 acetonitrile-water).

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creasing pH, is indicative of acid catalysis. This redox process does not appear to proceed through a free-radical mechanism as indicated by the galvinoxyl assay.[9]

Nine out of 13 anti-fluorescyl antibodies tested were able to accelerate the redox reaction described. The catalytic activity of one of these (66D2) was analyzed in detail. Saturation kinetics were observed with both sulfite (S) and **1**. Catalytic rates at several concentrations of the two substrates provide a complete set of data for extrapolation of kinetic constants from the double-reciprocal plots (Figure 1). A common x intercept suggests that the two sites are independent. Replots of the slopes and y intercepts yield the following values: $k_{cat} = 0.02\ s^{-1}$, $K_m^S = 3$ mM and $K_m^1 = 0.6\ \mu M$.[10]

The rate enhancement can be gauged by comparing the pseudo-second-order rate constant k_{cat}/K_m^1 (encounter of **1** with the Ab·SO_3^{2-} complex) with k_{uncat}, suggesting a factor of 3×10^5. For the encounter of sulfite with the Ab·**1** complex, the rate factor is 60. The antibody process is catalytic in a dilute, but useful, concentration range up to 0.2 M in either substrate.

Fluorescein was found to be a potent inhibitor of this reaction with a K_i of about 10^{-10} M obtained from a Henderson plot. This reflects the tight binding of fluorescein to 66D2 as measured by fluorescence quenching ($K_d = 1.1 \times 10^{-11}$ M).[5] Various small anions also inhibit the reaction; fluoride, chloride, bromide, and iodide (K_i = 34, 22, 19, and 13 mM, respectively), sulfate (9 mM), phosphate (9 mM), and benzoate (9 mM). These can presumably fill the benzoate subsite, which is known to bind a variety of small carboxylate species.[5]

The pH dependence of the catalytic reaction is sigmoidal with a maximum at acidic pH and an inflection point at about pH 6.7 (Figure 2). This may be due to participation of acidic group(s) on the protein with a pK_a near neutrality. Chemical modifications targeting histidine, arginine, and tyrosine reduced the catalytic activity by 50, 85, and 95%, respectively.[11]

The binding of reazurin[10] and that of resorufin[5] to 66D2 are very similar, and therefore the effect of this antibody does not appear to be in altering the redox potential of the dye as observed for flavin-binding antibodies.[12] The avid binding of the dye is intriguing as the catalyst is saturated at very low concentrations. The rate may be limited by the dissociation of product from the active site. Moreover, the efficient binding of substrates ($K_m^S K_m^1 = 2 \times 10^{-9}$ M) versus the apparent transition state binding ($k_{uncat}/(k_{cat}/K_m^S K_m^1) = 1 \times 10^{-8}$ M) accounts for the low catalytic efficiency at higher concentrations (effective molarity = 0.2 M).

The anti-fluorescyl immune response is instructive as a model for multisubstrate binding. Antibodies capable of binding to the benzoate fragment may appear only in the mature response.[13] Though the two sites appear to be independent, further studies are necessary to determine if the mechanism of binding is ordered. Molecular recognition at both subsites of the antibody is due in large part to interactions at ionic or polar residues.[5] The role of electrostatic interactions in antibody–antigen complexation[14] and in enzyme catalysis[15] has been appreciated. Accumulating evidence suggests that strategically placed ionic residues in a hapten can induce antibodies with enzyme-like combining sites.[4]

Future efforts toward developing antibodies as redox catalysts should focus on the transformation of specific organic substrates as well as on the integration of recyclable cofactors. Further understanding of factors that influence the formation of binding sites with multisubstrate complementarity will also prove useful for applications of antibody catalysts to diverse bimolecular reactions.[16]

(9) Greene, F. D.; Adam, W.; Cantrill, J. E. *J. Am. Chem. Soc.* **1961**, *83*, 3461.

(10) The dissociation constant of resazurin from 66D2 has been determined independently by the fluorescence quenching technique[5] ($K_d = 5 \times 10^{-8}$ M). An order of magnitude difference between this value and the observed K_m indicates that turnover is faster than the dissociation of **1** from 66D2.

(11) Selective amino acid modifications were carried out at pH 8 with diethyl pyrocarbonate (histidine), phenylglyoxal (arginine), and tetranitromethane (tyrosine) added in about 100-fold excess over the antibody.

(12) Shokat, K. M.; Leumann, C. J.; Sugasawara, R.; Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1172.

(13) Gollogly, J. R.; Cathou, R. E. *J. Immunol.* **1974**, *113*, 1457.

(14) Getzoff, E. D.; Tainer, J. A.; Lerner, R. A.; Geysen, H. M. *Adv. Immunol.* **1989**, *43*, 1.

(15) Warshel, A.; Naray-Szabo, G.; Sussman, F.; Hwang, J.-K. *Biochemistry* **1989**, *28*, 3629.

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Reaction of Trimethylaluminum with Carbon Monoxide in Low-Temperature Matrices

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The interaction of carbon monoxide with aluminum has been characterized in infrared, ESR, chemiluminescence, and EELS studies. Ogden and co-workers[1] obtained the infrared spectrum of a species identified as $Al_x(CO)_2$ with a CO stretch at 1988 cm^{-1}. Chevier et al.[2] report bands at 1985 and 1904 cm^{-1} for $Al(CO)_2$ and suggest that other bands may be due to Al_3CO and $Al_2(CO)_4$. An ESR study by Kasai and Jones[3] identified $Al(CO)_2$ formed in solid argon by cocondensation of Al atoms and CO. Analysis of chemiluminescence from the gas-phase reaction of Al, CO, and O_3 by Gole and co-workers[4] provides an estimate of the binding energy of $Al(CO)_2$ of 0.7 eV. CO adsorbed on a clean Al(100) surface exhibits a band at 2060 cm^{-1} in the electron energy loss spectrum.[5]

Our findings that organoaluminum compounds markedly attenuate the carbonylation of organomagnesium and organolithium reagents have prompted us to study the reaction between trimethylaluminum (TMA) and CO at 15–35 K. We have examined the infrared spectrum of the species formed upon cocondensation of trimethylaluminum and carbon monoxide in argon matrices. The data are consistent with the formation of a weakly bound complex $(CH_3)_3Al{<}CO$. The reaction of TMA with carbon monoxide was studied in solid argon at temperatures from 15 to 35 K. Gas mixtures of 0.2–1% CO and less than 1% TMA in argon were deposited on a CsI window cooled by a closed-cycle helium refrigerator. Infrared spectra were recorded at various stages of warming the matrix to allow diffusion of the reactants. These spectra were compared to those of TMA as well as CO individually on argon matrices. A new infrared band appeared at 2185 cm^{-1} when both reagents were deposited in the matrix. This spectrum is shown in Figure 1. We attribute this band to the CO stretching vibration of the complex. This vibration is 47 cm^{-1} higher than that of uncomplexed CO in the matrix.

No other IR bands attributable to the complex were observed. When ^{13}CO is used, the band shows the expected shift to 2134 cm^{-1}. Passing the gases through a heated quartz tube in the vacuum system just before deposition increases the area of the 2185-cm^{-1} band relative to the 2140-cm^{-1} band of free CO. The infrared spectra of both monomer and dimer of TMA have been thoroughly studied in argon matrices by Kvisle and Rytter.[6] They

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(1) Hinchcliffe, A. J.; Ogden, J. S.; Oswald, D. D. *J. Chem. Soc., Chem. Commun.* **1972**, 338.

(2) Chemier, J. H. B.; Hampson, C. A.; Howard, J. A.; Mile, B. *J. Chem. Soc., Chem. Commun.* **1986**, 730.

(3) Kasai, P. H.; Jones, P. M. *J. Am. Chem. Soc.* **1984**, *106*, 8018.

(4) McQuaid, M.; Woodward, J. R.; Gole, J. L. *J. Phys. Chem.* **1988**, *92*, 252.

(5) Paul, J.; Hoffman, F. M. *Chem. Phys. Lett.* **1986**, *130*, 160.

0002-7863/89/1511-9110$01.50/0