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.

(16) This work was supported by Grant GM 35318 from the National Institutes of Health. N.J. acknowledges with thanks a postdoctoral fellowship from the Cancer Research Institute.

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 $(CH_3)_3Al{\leftarrow}CO$. 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

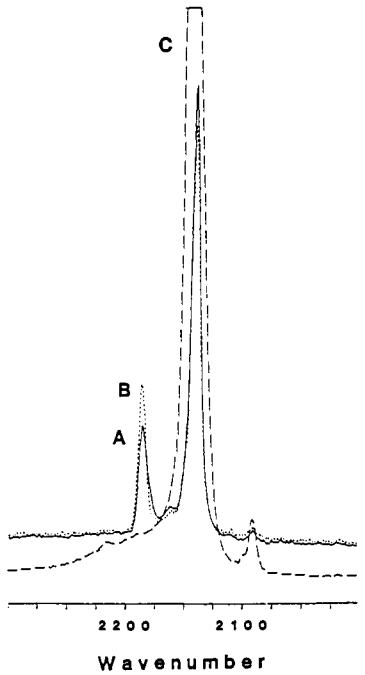

Figure 1. Infrared spectrum of trimethylaluminum and carbon monoxide. A: TMA + CO in argon. B: After warming matrix to allow migration. C: CO without TMA.

report a monomer:dimer ratio of 7:1 with no heating of the gas prior to deposition and an increase to >200:1 when the gas is heated to 350 °C.

Attempts to observe the complex at room temperature using an IBM 95 FTIR instrument with 0.25-cm^{-1} resolution revealed no observable band at 2185 cm^{-1}. Ab initio calculations indicate that the oscillator strengths for complexed CO and free CO are nearly identical. With the sensitivity of this instrument at pressures of 0.1 atm for CO and 0.01 atm for $AlMe_3$, we would detect the complex if the equilibrium constant for complex formation were 1 or greater. Assuming $\Delta S \approx -40$ cal K^{-1} mol^{-1} for formation of the complex gives, as an upper limit for the exothermicity of complex formation, $\Delta H = -12$ kcal mol^{-1}. We did not observe any ultraviolet absorption above 200 nm in experiments carried out on a similar apparatus using a sapphire window.

In order to gain further insight into the nature of the aluminum–carbon monoxide bonding, MNDO and ab initio calculations were carried out with different substituents bonded to aluminum. Using the MOPAC4 program, several features of the reaction system were calculated: (1) the enthalpy change associated with formation of the complex; (2) the structure of the complex; (3) the vibrational frequencies and intensities; and (4) charge distribution.

These computational results indicate that as carbon monoxide approaches the aluminum atom in TMA from a distance of 4.0 Å, the energy decreases with a barrier less than 1 kcal mol^{-1} to a minimum at a distance of 1.86 Å with an overall decrease in energy of 28 kcal mol^{-1}. The angle from CH_3 to Al to CO is 104°, as would be expected from theory when the aluminum atom accepts the lone pair from CO and rehybridizes toward sp^3. The most intense vibration was calculated to be the CO stretch at 2430 cm^{-1}. By comparison, the stretch in free CO was calculated to be 2382 cm^{-1}. The formation of the complex results in an increase in wavenumber of the CO stretch of 48 cm^{-1}.

The ab initio calculations of AlH_3–CO at the 6-31G* level gave similar results. The calculated binding energy of CO to trimethylaluminum is 7 kcal mol^{-1}, which is substantially less than the value calculated by MNDO. The calculated Al–CO bond length is 2.37 Å. The calculated CO stretching wavenumber is 2506 cm^{-1} compared to a calculated value of 2438 cm^{-1} for free CO: an increase of 68 cm^{-1}.

(6) Kvisle, S.; Rytter, E. *J. Mol. Struct.* **1984**, *117*, 51.

The increase of the CO stretching frequency upon complex formation with TMA is an indication of a difference in the bonding compared with that of CO bonded to Al atoms or surfaces. In the latter cases, P_π–π^* bonding is significant and contributes to a weakening of the CO bond.[7] In the case of the complex with TMA, the interaction is primarily that of the lone pair on carbon, the 5σ orbital of CO, occupying the vacant orbital on aluminum. Removal of electron density from this orbital on CO, which is weakly antibonding, results in an increase in the CO bond strength. This effect is noted in both the ab initio and MNDO calculations.

A similar increase in CO stretching frequency is found in BH_3·CO, which has a CO stretch at 2165 cm^{-1} in the gas phase.[8] Thus, both BH_3 and $Al(CH_3)_3$ complexes have little contribution to bonding from P_π–π^* interaction.

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(7) (a) McIntosh, D.; Ozin, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 3167. (b) Bagus, P. S.; Nelin, C. J.; Bauschlicher, C. W. *Phys. Rev. B* **1983**, *28*, 5423.
(8) Bethke, G. W.; Wilson, M. K. *J. Chem. Phys.* **1957**, *26*, 1118.

5′-{[(*Z*)-4-Amino-2-butenyl]methylamino}-5′-deoxyadenosine: A Potent Enzyme-Activated Irreversible Inhibitor of *S*-Adenosyl-L-methionine Decarboxylase from *Escherichia coli*

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The polyamine biosynthetic pathway is an important target for the design of chemotherapeutic agents.[1] The actual rate-limiting step in the formation of spermidine and spermine is catalyzed by *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50, AdoMetDC), a pyruvoyl-containing enzyme.[2] A number of reversible and irreversible potent AdoMetDC inhibitors have been reported.[3] However, they were neither sufficiently potent, nor sufficiently stable, nor sufficiently selective to permit investigation of AdoMetDC importance in cellular physiology in vivo.[3] An enzyme-activated irreversible inhibitor may fulfill these requirements.

The mechanism of *Escherichia coli* AdoMetDC inactivation by AdoMet was recently demonstrated.[4] It involves the transamination of the nascent reaction product to the pyruvoyl group, followed by the elimination of methylthioadenosine and the generation of the Michael acceptor 2-propenal, which could alkylate a nucleophilic residue in the active site. Furthermore, it has been recognized that analogues of decarboxylated AdoMet with a nitrogen atom in place of the sulfur produced also some time-dependent inhibition of mammalian AdoMetDC.[5]

On the basis of these data, we have designed 5′-{[(*Z*)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine (**1**) as a potential enzyme-activated irreversible inhibitor of AdoMetDC.

(1) (a) McCann, P. P.; Pegg, A. E.; Sjoerdsma, A. *Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies*; Academic Press: Orlando, FL, 1987. (b) Pegg, A. E.; McCann, P. P. *Am. J. Physiol.* **1982**, *243*, C212–C221.
(2) (a) Snell, E. E. *Adv. Exp. Med. Biol.* **1982**, *148*, 257–272. (b) Williams-Ashman, H. G.; Pegg, A. E. In *Polyamines in Biology and Medicine*; Morris, D. R., Marton, L. J.; Eds.; Marcel Dekker, Inc.: New York and Basel, 1981; pp 43–73. (c) Markham, G. D.; Tabor, C. W.; Tabor, H. *J. Biol. Chem.* **1982**, *257*, 12063–12068.
(3) Pegg, A. E. *Cancer Res.* **1988**, *48*, 759–774.
(4) Anton, D. L.; Kutny, R. *Biochemistry* **1987**, *26*, 6444–6447.
(5) Kolb, M.; Danzin, C.; Barth, J.; Claverie, N. *J. Med. Chem.* **1982**, *25*, 550–556.

0002-7863/89/1511-9111$01.50/0