for M = Zn^{2+} and Cd^{2+} was within the error margins, a clearer distinction was made through a further direct competition experiment. Transfer of a film of 7Fe Fd III into buffer electrolyte solutions containing a equal concentration of Zn^{2+} and Cd^{2+} (1-5 μM in each, prepared by dilution of a concentrated 1/1 stock solution) produced, after equilibration at -300 mV, voltammograms that were dominated by D' signals due to $[Cd3Fe\text{-}4S]^{2+/+}$. When the Zn/Cd stoichiometry was raised to 2/1, D' signals due to $[Zn3Fe\text{-}4S]^{2+/+}$ and $[Cd3Fe\text{-}4S]^{2+/+}$ appeared at approximately equal amplitude. Thus, we may conclude that the order of K_d values is as expected[28] for a divalent metal ion coordinated to a sulfide-rich site; that is, $Cd^{2+} \geq Zn^{2+} \gg Fe^{2+}$.

For a wider evaluation, analogous experiments were carried out on the [3Fe-4S],[4Fe-4S] ferredoxin I from *Azotobacter chroococcum*. This protein also gave a stable film displaying three pairs of waves. However, the form of the voltammogram was *unchanged* following a period of at least 30-min voltammetric cycling in solutions (pH 7) containing Fe^{2+} (3 mM), Zn^{2+} (3 mM), or Cd^{2+} (15 μM). This absence of reactivity of the 3Fe cluster in this protein was confirmed by experiments undertaken on a bulk solution.

The broad implication from these studies is that the voltammetry of a protein that is confined to an electrode surface as a molecular film (and is thus readily transferable between various solutions) can be extremely informative, providing an accurate reflection of complicated chemistry that occurs in bulk solution. As a surface electrochemical technique, it presents a highly sensitive and specific analytical method for viewing and controlling the chemistry of active sites in the time domain. In the example that we have described, the formation of specific cluster species, undefined by all but sophisticated low-temperature spectroscopic methods, is clearly observed through the appearance of well-defined voltammetric signals. As a quantitative tool for the determination of equilibrium and kinetic parameters, the approach should permit the investigation of a rich and complex chemistry associated with labile clusters. The results show that, in Fd III, the $[3Fe\text{-}4S]^0$ cluster readily binds additional divalent metal ions in an equilibrium process that is rapidly established, with an affinity order $Cd^{2+} \geq Zn^{2+} \gg Fe^{2+}$. Furthermore, the binding of such metal ions to produce $[M3Fe\text{-}4S]^{2+}$ is relatively weak, and the $\{[M3Fe\text{-}4S]\}/\{[3Fe\text{-}4S]\}$ population ratio can be effectively modulated by variations in the M^{2+} identity and its activity across a range 10^{-4}-10^{-6} M. Little is known about the relative available concentrations of Fe^{2+} and Zn^{2+} within the cells of obligate anaerobic bacteria such as *D. africanus*. However, if these conclusions prove to be more general, it is important to be aware of the possibility that heterometal-sulfur clusters of the type described here may exist naturally in some proteins. Furthermore, the demonstrated capability for rapid interconversion equilibria might be exploited for some regulatory purpose. The latter possibility is indeed suggested in the recent report by Rouault et al.[29] concerning the sequence similarity between aconitase and a mRNA binding protein—the "iron-responsive element binding protein" (IRE-BP)—that links the expression of ferritin and of the transferrin receptor to Fe levels in the cell.

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Registry No. Fe^{2+}, 7439-89-6; Zn^{2+}, 7440-66-6; Cd^{2+}, 7440-43-9.

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Communications to the Editor

Antibody-Catalyzed Bimolecular Imine Formation

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Recently chemists have enlisted the cellular machinery of the immune system to produce highly selective catalysts.[1-3] One of several strategies for generating catalytic antibodies is the use of antibody affinity and specificity to bind both substrate and a cofactor in a catalytically productive orientation. Pyridoxal phosphate (PLP) is a versatile enzymatic cofactor which is commonly involved in reactions of α-amino acids involving carbanion intermediates.[4] The reactions catalyzed by this cofactor include transamination, decarboxylation, racemization, and β- and γ-elimination. Transamination, in particular, is a well-studied reaction, both by enzymologists[5-8] and by organic chemists.[9-11] The mechanistic interest in this reaction as well as the importance of chiral α-amino acids in biological studies and as synthetic intermediates suggest transamination as a target for antibody catalysis.

One of the earliest attempts to generate catalytic antibodies was reported by Raso and Stollar, who, in 1975, generated antibodies to a reduced aldimine formed from 3-aminotyrosine and PLP.[12-14] Although these polyclonal antibodies did not display any significant catalytic properties, it was demonstrated that amino acid, PLP, and aldimine were bound by the antibody. We prepared hapten (**1**) via reductive amination of pyridoxal with L-*p*-nitrophenylalanine and sodium borohydride. The 5'-hydroxyl group of the cofactor was then converted to a thiol via the isothiuronium salt, activated with 2,2'-dithiodipyridine, and

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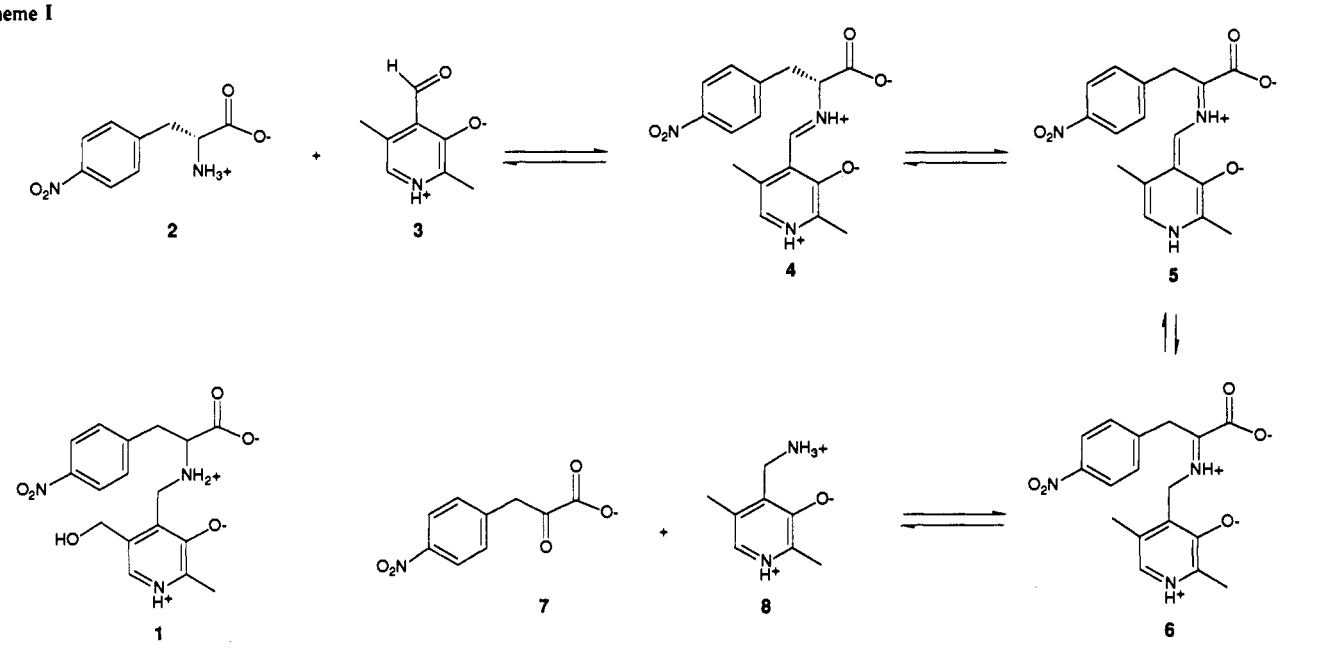
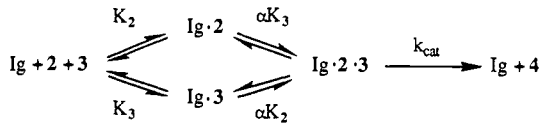
Scheme I



coupled to thiolated carrier protein[15] by disulfide exchange. Monoclonal antibodies were produced and purified by standard protocols.[16]

Antibodies specific for hapten **1** might be expected to catalyze imine formation by approximation of reactants in the antibody combining site. In addition, C-4′ of the hapten is tetrahedral, as it is in the aminal intermediate formed by addition of amine to aldehyde. An sp^3 center (a secondary alcohol) has proven effective in eliciting antibodies which catalyze reactions involving similar tetrahedral transition states.[17] Seven monoclonal antibodies specific for hapten **1** were assayed as catalysts for aldimine formation (Scheme I). Antibody 17C5-11C2 catalyzed aldimine formation from D-*p*-nitrophenylalanine (**2**) and 5′-deoxypyridoxal (**3**) in 50 mM potassium phosphate, 50 mM sodium chloride, pH 7.0. The kinetics of the antibody-catalyzed reaction are consistent with a random-binding mechanism:[18]



The initial velocity data at five fixed concentrations of **2** and varied concentrations of **3** yield a family of Lineweaver–Burk plots (Figure 1). The slopes and *y*-intercepts are then plotted as a function of the inverse amino acid concentration (Figure 1 inset) to determine the kinetic constants: k_{cat} = 18 min^{-1}, K_2 = 0.12 mM, K_3 = 0.71 mM, and α = 2.4. The second-order rate constant for the uncatalyzed reaction is 85 M^{-1} min^{-1}. A kinetic analysis of aldimine formation from L-*p*-nitrophenylalanine in the presence of antibody indicated little or no specific catalysis, in contrast to that observed with the D-enantiomer.

The antibody-catalyzed reaction was completely inhibited by the addition of 50 μM hapten (**1**). Interestingly, both D- and L-isomers were effective inhibitors. Fluorescence quenching experiments performed under conditions identical to those of the kinetic assays showed the L-enantiomer to be the tighter binding of the two isomers (L, K_d = 6 nM; D, K_d = 17 nM). The inability of the antibody to catalyze aldimine formation from L-*p*-nitrophenylalanine suggests that the antibody discriminates the diastereomeric transition states for aminal formation or subsequent dehydration better than it discriminates enantiomers of hapten (**1**).

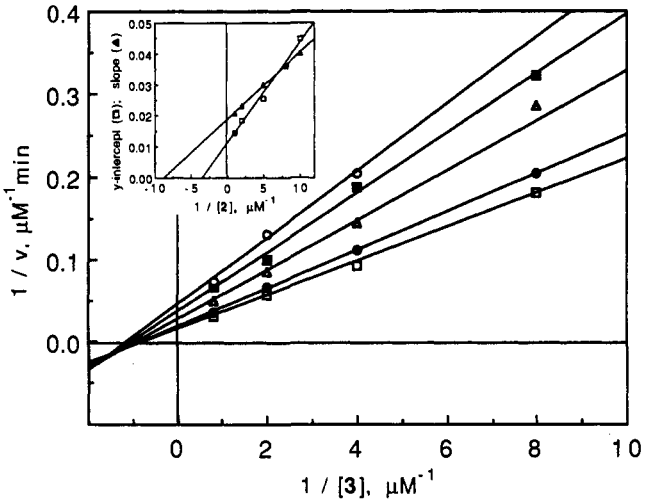


Figure 1. Lineweaver–Burk plots for imine formation as a function of 5′-deoxypyridoxal (**3**) at various fixed concentrations of D-*p*-nitrophenylalanine: (□) 1 mM, (●) 500 μM, (△) 200 μM, (■) 125 μM, and (○) 100 μM. Inset: replot of slopes (▲) and *y*-intercepts (□) of Lineweaver–Burk plots as a function of [3]. Assays were conducted in 50 mM potassium phosphate, 50 mM sodium chloride buffer, pH 7.0 at 25 °C. The antibody concentration was 5 μM. The reaction was monitored at 430 nm ($\Delta\epsilon$ = 4500 M^{-1} cm^{-1}) with a Kontron Instruments Uvikon 860 UV-visible spectrophotometer.

It is conceivable that antibodies specific for **1** might catalyze transamination or other PLP-dependent reactions through proton transfer by a hapten-induced active site base.[19,20] However, none of the antibodies significantly catalyzed transamination of either **7** and **8** or of **2** and **3** (in phosphate or imidazole buffers, or in the presence of metal ions). The antibodies also failed to catalyze 5′-deoxypyridoxal-dependent racemization or decarboxylation of either D- or L-*p*-nitrophenylalanine. These results are not surprising since the design of our hapten did not allow for the hybridization changes accompanying transamination, i.e., the planarity of the delocalized carbanion intermediate (**5**). Stabilization

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of this fully conjugated intermediate in addition to introduction of an active site base is likely to be crucial in successful antibody catalysis of the proton transfer. Such an active site base might be introduced either by hapten design or by attaching a base to the cofactor.[11]

Antibody-cofactor catalysis of enantioselective amino acid synthesis remains an attractive goal. This study demonstrates the feasibility of the initial step of PLP catalysis: stereospecific formation of an aldimine intermediate. Improvements in hapten design coupled with mutagenesis or genetic selections may yield antibodies with the desired catalytic capabilities.

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Supplementary Material Available: Experimental details for the preparation of **1-11**, kinetic assays, and determination of antibody-inhibitor dissociation constants (11 pages). Ordering information is given on any current masthead page.

Chiral Dihydropyridones as Synthetic Intermediates. Asymmetric Synthesis of (+)-Elaeokanine A and (+)-Elaeokanine C

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Since their isolation by Jones and co-workers,[1] the *Elaeocarpus* alkaloids have received considerable attention as targets for synthesis. Elaeokanine A (**1**) has been prepared several times in racemic form[2] and once enantioselectively.[3] The synthesis of (±)-elaeokanine C (**2**) has also been carried out in several laboratories,[4] but an enantioselective preparation has not been previously reported and the absolute configuration of the natural product was unknown. As part of a program directed at developing the utility of 1-acyldihydropyridines and 1-acyldihydropyridones as synthetic intermediates,[5] we explored a strategy for the enantioselective synthesis of the elaeokanines A and C that was based on our recently developed asymmetric synthesis of 2-alkyl-2,3-dihydro-4-pyridones.[6] Our synthetic plan followed the retrosynthetic analysis shown in Figure 1.

Reaction of chiral 1-acylpyridinium salt **3**, prepared in situ from 4-methoxy-3-(triisopropylsilyl)pyridine[6] and the chloroformate of (-)-8-(4-phenoxyphenyl)menthol,[7] with Grignard reagent **4**[8]

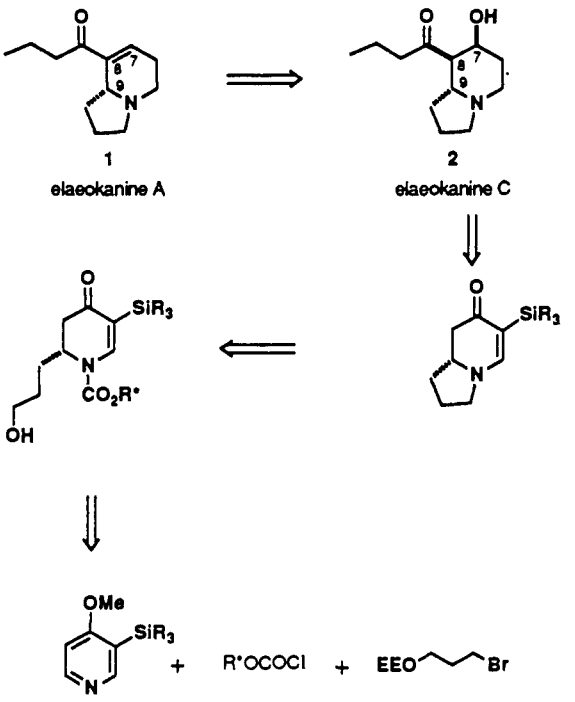
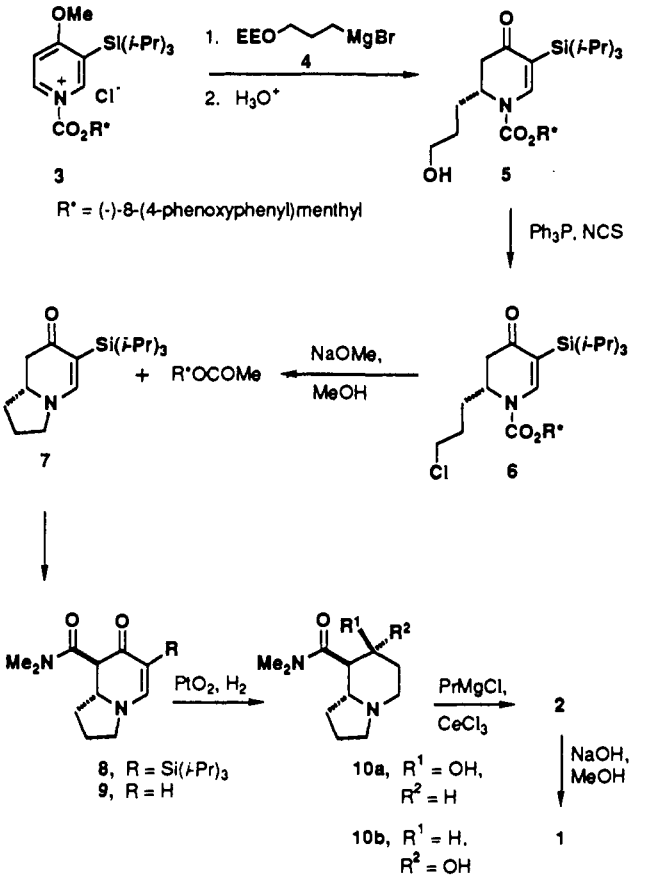


Figure 1.

in THF/toluene at -78 °C gave the alcohol **5** in 82% crude yield and 94% de. After purification by chromatography (silica gel, 10% EtOAc/hexane), alcohol **5** (65% yield) was converted to chloride **6**, mp 81-83 °C, in 89% yield by treatment with triphenylphosphine and *N*-chlorosuccinimide.[9] On removal of the



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