In conclusion, the solution conformation and the molecular structure in the solid state of the [3]-catenand **1** are in good agreement. The geometry of the molecular system (ternary structure) is determined by the formation of optimal intramolecular associations between aromatic subunits, the contribution of intermolecular stacking being also significant in the crystal.

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Supplementary Material Available: Tables of atomic coordinates, bond distances and angles, anisotropic thermal parameters, relevant distances in one whole molecule, and relevant dihedral angles between mean planes (9 pages); tables of observed and calculated structure factors (15 pages). Ordering information is given on any current masthead page.

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Antibody Catalysis in Reverse Micelles

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Recently, it was demonstrated that the high affinity and specificity of antibodies could be exploited in the design of catalysts for acyl-transfer,[1-3] pericyclic,[4] photochemical,[5] and redox reactions.[6] Because antibodies can be selectively elicited to a vast array of structurally diverse molecules,[7] these experiments offer the possibility of producing tailor-made catalysts for applications in biology, chemistry, and medicine.[8] The versatility of antibody catalysis would be substantially expanded if reactions could also be performed in nearly anhydrous solvents, reverse micelles, or aqueous-organic biphasic systems.

Several low molecular weight proteins have been solubilized in the hydrophilic core of reverse micelles, which are formed when water/surfactant mixtures are dissolved in water-immiscible solvents.[9-11] NMR,[12] CD, and fluorescence[13] studies have demonstrated that these proteins do not undergo major structural changes when solubilized in reverse micelles and kinetic studies reveal that enzymatic activity is also retained.[14] Quasielastic light[15] and small-angle neutron scattering probes[11,16] of reverse micelle structure demonstrated that the radii of reverse micelles are similar to the radius of the enclosed macromolecule. The size of reverse micelles is also controlled by the molar ratio of water to detergent (Wo). Most low molecular weight enzymes display maximal activity at Wo's between 10 and 15,[16] whereas lipoxygenase (100 K daltons) displayed maximal activity at a Wo of 30.[10] There is only one report describing antigen–antibody interactions in reverse micelles,[17] and in that case polyclonal antibodies were used and antibody activity was assessed indirectly. We now report that an antibody generated to the transition-state analogue, phenylphosphonate **1**, catalyzes the hydrolysis of phenylacetate in reverse micelles with rates comparable to those observed in aqueous solutions.

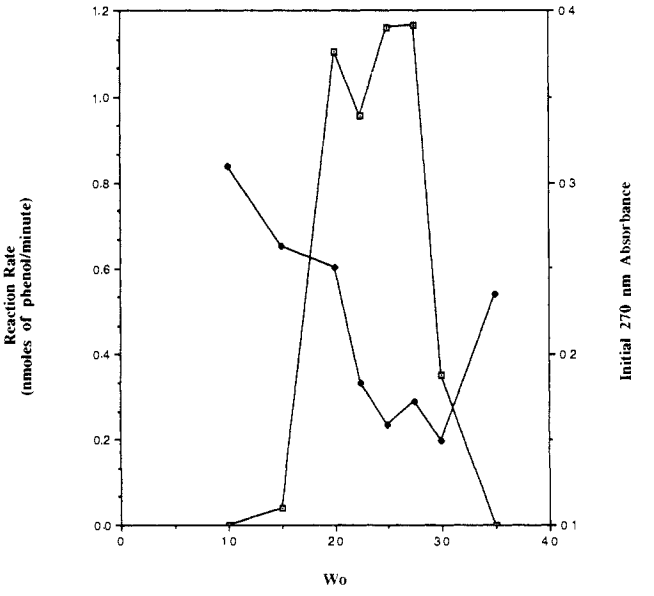
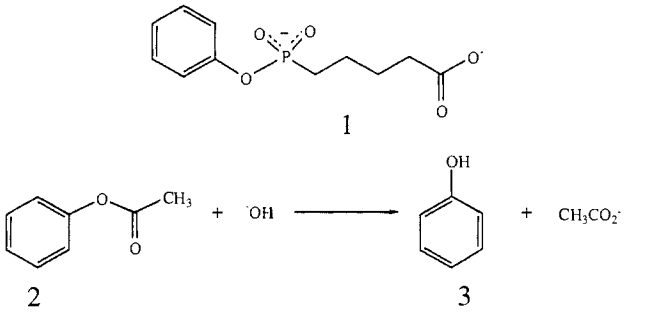


Figure 1. Rate of antibody-catalyzed hydrolysis of phenylacetate in reverse micelles as a function of Wo (□). The extent of forming antibody-containing reverse micelles (i.e., the initial A_{270}) as a function of Wo (◆). Note that the 270-nm absorbance reflects contributions from **2**, protein, and reverse micelle turbidity.

Scheme I



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Phenylphosphonate **1** was synthesized in five steps from triethyl phosphite and methyl 5-bromopentanoate.[18] Phosphonate **1** was coupled to the carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) in dilute aqueous HCl, pH 5.0, with 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide, followed by exhaustive dialysis against aqueous 10 mM phosphate, 150 mM NaCl buffer, pH 7.4. Quantitation of the hapten/carrier ratio afforded ratios in the range 15:1. BALB/c mice were immunized with the KLH-phosphonate conjugate emulsified in complete Freund's adjuvant. A fusion with Sp2/0 myeloma as the fusion partner was performed by the standard methods,[1] and 13 IgG's were purified from ascitic fluid by affinity chromatography on protein A-coupled Sepharose 4B[1] and dialyzed exhaustively against reaction buffer (20 mM Tris·HCl, 20 mM NaCl, pH 8.5). The homogeneity of antibodies was determined by HPLC.[19]

The rates of hydrolysis of phenylacetate **2** in the presence (k_{obsd}) and absence (k_{uncat}) of antibody were determined at 35 °C, pH 8.5.[20] Of the 13 antibodies isolated, five (38%) were found to be catalytic. Lineweaver-Burke analyses of the antibody-catalyzed reactions afforded values of k_{cat} between 0.3 and 18.8 min^{-1} and K_m values between 157 and 534 μM. The fact that a high percentage of those antibodies isolated were catalytic may reflect that the tetrahedral phosphonate is a dominant structural and recognition element of this simple hapten. The IgG 20G9 has a k_{cat} of 18.8 min^{-1} and a K_m of 157 μM, which corresponds to a rate constant enhancement factor of 14 700 over the uncatalyzed reaction.[20] A K_i of 39 nM for phosphonate **1** was determined from Henderson plots[21] at 60 and 168 μM **2** for this antibody.

Antibody-containing micellar solutions were formed by injecting an aqueous antibody stock solution into an isooctane solution of 50 mM bis(2-ethylhexyl)sodium sulfosuccinate (AOT)/water.[23] Antibody-catalyzed hydrolysis of **2** by the antibody 20G9 was observed at Wo values between 21 and 31 (Figure 1), which is consistent with the Wo values forming the highest concentrations of reverse micelles as judged by UV absorbance. At a Wo of 23, Lineweaver-Burke analysis of the 20G9-catalyzed reaction afforded a k_{cat} of 3.89 min^{-1} and a K_m of 569 μM. While these results demonstrate retention of antibody activity in an isooctane bulk solution, detailed comparisons of catalytic rates in aqueous buffer and reverse micelles are complicated by the unknown pH and substrate concentrations inside the micelles. The changes in K_m and k_{cat} may also reflect the increased solubility of phenylacetate in isooctane relative to water (partition coefficient is 7), resistance to mass transfer across micellar boundaries, and/or possible changes in antibody structure within reverse micelles. Interestingly, spontaneous hydrolysis of phenylacetate **2** was not observed in reverse micelles containing buffer, a noncatalytic antibody, or hapten-inhibited catalytic antibody.

In conclusion, we have demonstrated that a catalytic antibody retains activity when solubilized within reverse micelles. Optimal antibody activity was observed at a Wo value of 28, consistent with the increased molecular weight of IgG molecules. Because the structures of antibodies are highly conserved, it is likely that most catalytic antibodies will also be active in reverse micelles. The ability of antibodies to function in reverse micelles should significantly expand the versatility of antibodies in catalysis.

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(18) Methyl 5-bromovalerate and triethylphosphite were heated to reflux for 14 h, and the resulting product was distilled to afford the phosphonate triester. The ester was hydrolyzed with concentrated HCl (100 °C), concentrated in vacuo, and converted to the acid chloride by treatment with thionyl chloride at reflux. The acid chloride was purified by distillation and subsequently converted to the phenyl triester by treatment with phenol (150 °C, 4 h). The ester was chromatographed on silica gel (Merck 60, 7:3 ether/hexanes) and subsequently hydrolyzed with 0.1 M aqueous NaOH (100 °C, 12 h). The reaction mixture was cooled, neutralized, applied to a DEAE-Sephadex column (HCO_3^- form), and eluted with a linear gradient of 0.0-1.0 M triethylammonium bicarbonate, pH 7.8. The phenylphosphonic acid was concentrated in vacuo, applied to a Dowex 50 (Li^+ form) column, and eluted with water to afford analytically pure hapten.

(19) Antibody was eluted from an ABX column (7.75 × 100 mm; J. T. Baker) with a linear gradient of 25 mM (2-[*N*-morpholino]ethanesulfonate (pH 5.7) to 1.0 M sodium acetate (pH 7.0) at 1.0 mL/min over 30 min.

(20) Initial rates were determined spectroscopically (270 nm) at pH 8.5 and 35 °C. Reactions were initiated by adding 50 μL of a 8.6 μM antibody stock solution to 1000 μL reaction buffer. Substrate concentrations (1% DMF final concentration) were determined spectroscopically (264 nm) and ranged from 42 μM to 1.40 mM. The uncatalyzed rate was determined between 5 and 40 mM Tris·HCl buffer concentrations (pH 8.5, 35 °C) and extrapolated to zero Tris·HCl, affording a value of k_{un} of 1.28×10^{-3} min^{-1}. Protein concentrations were determined via bicinchoninic acid method[22] and compared to a standard bovine IgG solution.

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(23) Antibodies were solubilized in micelles by either (a) adding 3.5 μL of a 185 μM stock antibody solution (20 mM NaCl, 20 mM Tris-HCl, pH 8.5) to 1000 μL of an isooctane solution containing 280 uM **2**, 50 mM AOT, and the appropriate concentration of water to yield Wo values between 11 and 36 or (b) adding 10 μL of a 70 μM stock antibody solution to 1000 μL of an isooctane solution containing 10 μL of water, 50 mM AOT, and varying concentrations of substrate.

Heme Model Studies Related to Cytochrome P-450 Reactions: Preparation of Iron-Porphyrin Complexes with Carbenes Bearing a β-Oxygen Atom and Their Transformation into Iron-*N*-Alkylporphyrins and Iron-Metallacyclic Complexes

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Evidence has been presented during this last decade for the formation of iron-carbene and iron-σ-alkyl complexes during the oxidation or reduction of several substrates by cytochrome P-450.[1] Model porphyrin complexes containing these iron-carbon bonds have been prepared and completely characterized.[1,2] The intermediate formation of such complexes has been recently postulated to interpret the hydrogen-deuterium exchange observed during *trans*-1-D-propene epoxidation by a rabbit liver cytochrome P-450 (Scheme I).[3]

Metallacyclic species of type A have also been proposed as possible intermediates in the formation of Fe—O—C—C—N *N*-alkylporphyrins of type C during oxidation of terminal alkenes by cytochrome P-450 and heme models.[4] The mechanism postulated in Scheme I involves the β-hydroxy-carbene complex B as a key intermediate that can form σ-alkyl complex A by intramolecular protonation at the carbenic carbon in a reversible process. These reactions have so far no precedent in the iron-porphyrin chemistry and raise several questions: (i) is it possible to prepare porphyrin-iron-carbene complexes with an oxygen atom in the β-position? (ii) are they transformed into σ-alkyl complexes by protonation? and (iii) do they lead to Fe—O—C—C—N

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