force-field methods but not with AM1. The latter method still seems to overstimulate to some extent steric interaction.

In conclusion, we recommend AM1 as the most reliable semiempirical MO method presently available for the structure optimization of sterically hindered hydrocarbons that contain a weakly coupled π-system.

Registry No. *cis*-Stilbene, 645-49-8; 1,2-diphenylcyclopropene, 24168-52-3; 1,2-diphenylcyclobutene, 3306-02-3; 1,2-diphenylcyclopentene, 1485-98-9; 1,2-diphenylcyclohexene, 41317-87-7.

Bait and Switch Strategy for Obtaining Catalytic Antibodies with Acyl-Transfer Capabilities

Kim D. Janda,* Michael I. Weinhouse, Diane M. Schloeder, and Richard A. Lerner*

Departments of Molecular Biology and Chemistry
Research Institute of Scripps Clinic
La Jolla, California 92037

Stephen J. Benkovic*

Department of Chemistry
Pennsylvania State University
University Park, Pennsylvania 16802

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Monoclonal antibodies have been shown to catalyze a variety of acyl-transfer reactions[1] by utilizing haptenic transition-state models.[2] In order for the scope and capabilities of these hydrolytic abzymes to be expanded, new strategies must be developed for eliciting catalytic activity in the combining sites of antibodies. Recent reports have focused attention on the modification of an antibody's binding pocket through either semisynthetic methods[3] or site-directed mutagenesis.[4] However, their generality may be reduced because of the lack of available structural data for catalytic antibodies. We felt that a process that could induce catalytically active groups de novo from our antigen might prove more advantageous because one can harness the vast variability of the immune response, via the somatic mutation process, to perform "in vivo" mutagenesis. Herein we report a tactic that elicits an amino acid (or acids) within the antibody's binding pocket to assist in an acyl-transfer reaction by a methodology we have previously termed "bait and switch" catalysis.[2]

Our plan involved the placement of a point charge within our antigen **1a** (Figure 1) in close proximity to the acyl moiety we wished to hydrolyze. The antibodies raised to this hapten should possess amino acid residue (or residues) at the binding site having a charge complementary to this haptenic charge.[5] In addition, the *N*-methylpyridinium salt **1a** will present to the antibody a hydroxylic group having a tetrahedral geometry that will serve as a representation of the acyl-transfer transition state. We intentionally left this position uncharged so that there would be no additional electrostatic effects. The benzoate substrate **3** (Figure 1) corresponding to hapten **1a** has similar steric dimensions (determined from MM2 calculations), but lacks the positive charge. Hence, the amino acid at the antibody binding site will be freed from ion-pair formation and can now serve as a potential general acid/base or nucleophilic catalyst. The pyridine hapten **2** will function as a control, since it is structurally identical with **1a**, but lacks a charge at physiological pH. Charge complementarity by Schultz et al. and Sugasawara has been previously employed to abstract a substrate proton in an antibody-catalyzed β-elimination reaction, although no comparison was made to a neutral hapten.[5d]

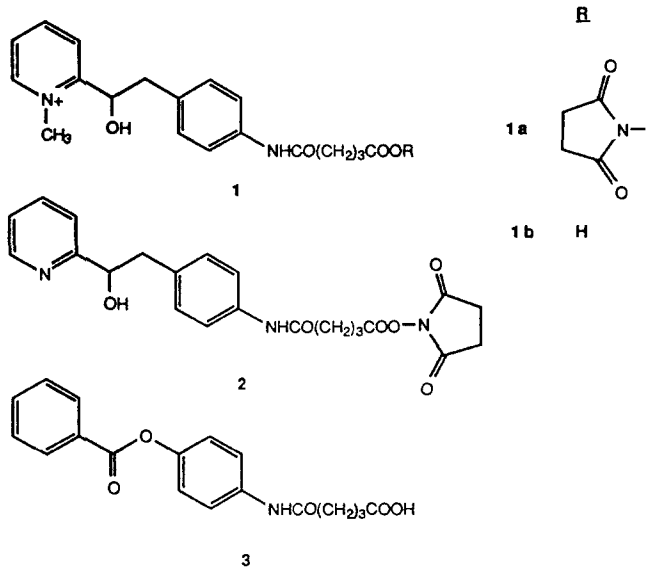

Figure 1. Structures of antigens (**1a**, **2**), inhibitor (**1b**), and substrate (**3**).

Hapten **1a** and **2** were synthesized in five and four steps, respectively, starting from 4-nitrophenethyl bromide.[6] Both **1a** and **2** were coupled (via the *N*-hydroxysuccinimide ester) to the carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). Balb/c mice were immunized with the KLH conjugate of **1a** and **2**, and antibodies were generated by standard protocols.[7] Immunization of **1a** produced 23 antibodies while hapten **2** yielded 21 hybridomas. All monoclonals were of the IgG class and were purified from ascites fluid by anion-exchange chromatography followed by affinity chromatography on a protein G column. Antibodies were judged to be homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.[8]

Antibodies at a concentration of 20 μM were initially screened (phosphate buffer 50 mM, pH 7.5, 100 mM NaCl, 37 °C) against benzoate ester **3** (500 μM) for the production of 5-[(4-hydroxyphenyl)amino]-5-oxopentanoic acid (**4**).[9] From the 23 monoclonal antibodies obtained to **1a**, *seven* were found to be catalytic; while *none* of the antibodies to hapten **2** showed any tendency to accelerate the rate of hydrolysis of ester **3**. The seven antibodies that were found to be catalytic were completely inhibited by the addition of free hapten **1b**. Such results suggest that catalysis follows binding of the substrate in the antibody binding pocket. Most significant was the overwhelming number of catalytic antibodies to hapten **1a** vs **2**. One of these seven catalytic antibodies was characterized in detail.

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(9) The analysis was performed via HPLC on an RP-C18 column eluting with water–acetonitrile (90:10) at a flow rate of 1 mL/min with UV detector set to 254 nm. The hydrolysis product, **4** (retention time 7 min), was collected and found to be identical by RP-HPLC coinjection and mass spectral analysis with an authentic sample.

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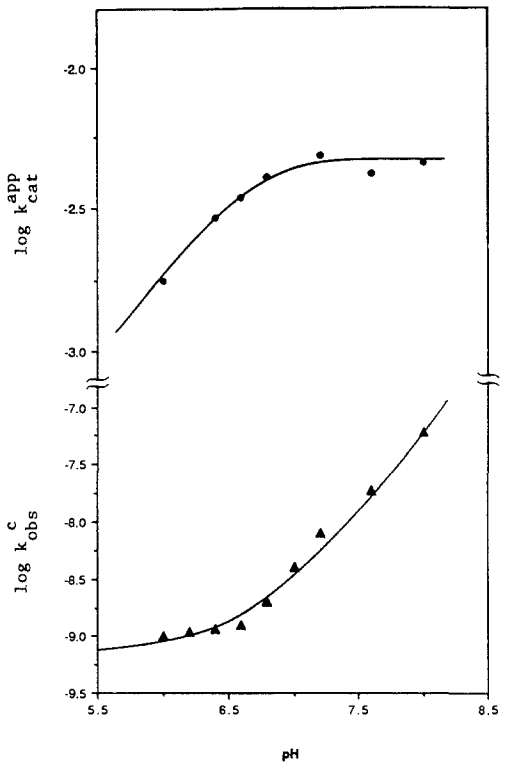

Figure 2. log k_{cat}^{app} vs pH plot (●) of antibody (30C6) catalyzed reaction of **3**. The calculated line was obtained by using $k_{cat}^{app} = k_{cat}[K_a/(K_a + a_H)]$. log k_{obsd}^{c} vs pH (▲) of **3** extrapolated to zero buffer concentration. The calculated line was obtained by using $k_{obsd}^{c} = k_0 + k_{OH^-}[OH^-]$. Values for k_{cat}, K_a, k_0, and k_{OH^-} are listed in the text.

The initial rate of hydrolysis of **3** (50 mM phosphate, 100 mM NaCl, pH 7.2, 37 °C) catalyzed by 30C6 (20 μM) followed Michaelis–Menten kinetics[10] with values of k_{cat}^{app} and K_m of (5 ± 0.2) × 10^{-3} min^{-1} and 1.12 ± 0.05 mM, respectively. The antibody-catalyzed hydrolysis of benzoate **3** was competitively inhibited (K_i = 83 ± 5 μM) by the addition of pyridinium salt **1b**. The pH dependence of the hydrolysis of **3** was examined in the presence of 30C6 (20 μM) between pH 6.0 and 7.2 (Bis-tris) and pH 7.2 and 8.0 (phosphate), both at 50 mM buffer and 100 mM NaCl, 37 °C (Figure 2). The pH dependence of k_{cat}^{app} reveals participation by the basic form of a dissociable group, whose pK_a was determined to be 6.26 ± 0.05 (Figure 2). Variation of the buffer ion concentration (12.5–50 mM) showed no dependency on k_{cat} on the presence of buffer species. For direct comparison, we measured the rates of hydrolysis (k_{obsd}^{c}) of **3** over the idential pH region extrapolated to zero buffer concentration (Figure 2). The pH rate profile implicated the species[11] involved in cleavage to be water in the pH region of 6.0–6.5 (k_0 = 0.6 × 10^{-9} min^{-1}) and hydroxide from pH 6.6 and above (k_{OH^-} = 4.2 × 10^{-2} min^{-1}). The ratio of k_{cat}/k_0, a comparison of the pH-independent antibody-catalyzed rate of **3** to that in water, corresponds to a rate acceleration by the antibody of over 1,000,000-fold. *Significantly, the pH optimum of the antibody-catalyzed reaction has been moved into the neutral pH region* by participation by as yet an unidentified amino acid.

We foresee an ensemble of multiple charges which might produce a number of catalytic groups giving us an additive rate effect. This possibility combined with access to a much larger repertoire of potential catalytic antibodies[12] improves the probability of developing superior catalysts.

(10) Concentrations of **4** were determined by HPLC measurements of its peak height relative to that of an internal standard over 1–2 h (three or more determinations). A standard curve showed linearity with concentrations of **4** up to 0.5 mM.

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Simple and Accurate Determination of Ion Translational Energy in Ion Cyclotron Resonance Mass Spectroscopy

Peter B. Grosshans,[†] Patrick Shields,[‡] and Alan G. Marshall*[,†,§]

Departments of Chemistry, Biochemistry, and Physics
The Ohio State University
120 West 18th Avenue, Columbus, Ohio 43210

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Fourier transform ion cyclotron resonance (FT/ICR) mass spectrometry is the most versatile and widely used technique for analysis of gas-phase ion/molecule reaction pathways, kinetics, equilibria, and energetics and is also suitable for a wide range of analytical applications, as summarized in numerous recent reviews.[1-12] A powerful tool in such studies is the collision-induced dissociation (CID, also known as CAD, collision-activated dissociation) technique, in which ions are first accelerated to higher translational energy (in this case, by increase in ICR orbital radius in an ICR ion trap);[8,13] subsequent ion-neutral collisions (e.g., with Ar atoms) then result in fragmentation of the parent ion to give daughter ions. The structure of the parent ion may then be inferred from the masses of the daughter ions[14,15] and/or the neutral fragments lost.[15] From the *radius r*, of that ICR orbit, the ion orbital translational *energy* is readily found:[16]

$$KE = \frac{q^2 B_0^2 r^2}{2m} \quad \text{(SI units)} \qquad (1)$$

in which q is ionic charge, m is ionic mass, and B_0 is the applied magnetic field strength. For example, the energy threshold for collision-induced dissociation may be determined from eq 1 by increasing the ICR radius (and thus ion translational energy) until the desired collision-induced dissociation is observed.[8,13]

Up to now, the translational energy of the accelerated ions in such experiments was computed from the measured radio-frequency (rf) excitation voltage amplitude and duration. Unfortunately, the equation on which all such prior computations have been based is valid only for electrodes of *infinte* extent[17] and

[†] Department of Chemistry.

[‡] Department of Physics.

[§] Department of Biochemistry.

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