A Highly Specific Metal-Activated Catalytic Antibody

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Strategies to induce catalytic antibodies are increasing at a rapid rate.[1] One important tactic for the induction of catalytic antibodies is the use of haptens which induce strategically placed amino acid residues in the antibody combining site. When these amino acid residues interact with functionalities that differ between the hapten and substrate, the process is termed "bait and switch" catalysis.[2,3] This process could be further advanced by designing haptens which induce cofactor binding sites in antibodies so that a combination of functionalities operating in a synergistic manner could provide routes to more sophisticated and efficient catalysts. Herein, we report our initial efforts on this multifaceted approach for catalytic antibody production.

Metal ion-promoted catalysis has been observed by Bruice and Fife and their colleagues[4] in ester and anhydride hydrolysis when there are functional groups present within the substrate's structure which can affix the metal ion in close proximity to the reactive moiety. A number of variables control the rate of catalysis in these reactions besides proximity effects, including strength of metal ion-binding to the reactant and metal ion-promoted OH^- attack. We reasoned that an antibody combining site may display similar properties if hapten **1**[2c] was employed as the immunogen. The structural features which make **1** appealing are the following. (1) A positively charged pyridinium moiety which has been shown to induce carboxylate residues in antibody combining sites.[2] Such residues might act as ligands which could enhance the binding of potential metal ions in the antibody binding site. (2) A hydroxyethylene isosteric replacement for the carboxylic ester moiety to be hydrolyzed. The hydroxyethylene functionality represents the acyl carbonyl and its developing tetrahedral transition state without imposing any electrostatic effects. (3) A methyl moiety on the pyridinium salt is present which gives some spatial latitude to accommodate a metal ion in the binding cleft. The net effect of this strategy is to force immunoglobulin binding to distinct metal entities rather than multidentate metal complexes.[5]

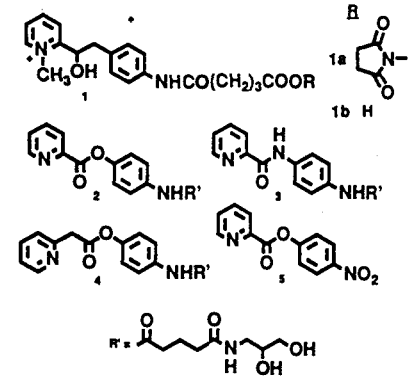

Figure 1. Structure of antigen (**1a**), inhibitor (**1b**), and substrates tested (**2–5**).

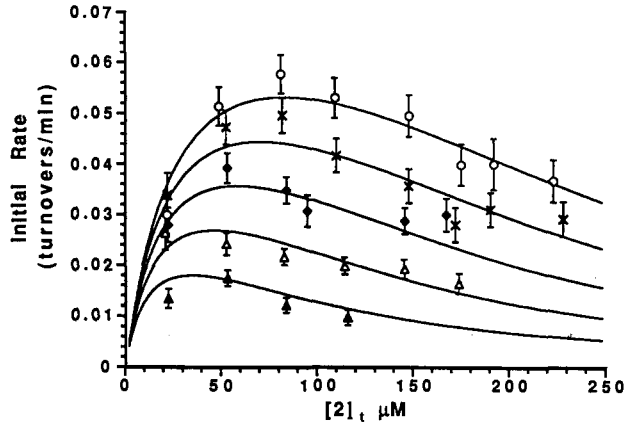

Figure 2. Initial rates of IgG 84A3-catalyzed reaction. Assay conditions: 12.5 mM MOPS, 75 mM NaCl, pH 7.0 at 25 °C. The antibody concentration is 10 μM for $[2]_t > 60$ μM and 5 μM for the other points. Error bars represent a 5% error for each measurement. Data points: (from top) ○ 300 μM Zn, × 250 μM Zn, ◆ 200 μM Zn, △ 150 μM Zn, ▲ 100 μM Zn. The lines were calculated as a simultaneous fit of all points using eq 1.

Pyridine ester **2**[6] (Figure 1) was screened as a potential substrate in the presence of divalent metal ions. We anticipated that metals would be weakly coordinated to the pyridine moiety of **2**, and it was hoped that the antibody would accept and anchor this metal complex in the antibody combining site. Twenty-three antibodies were analyzed. One antibody 84A3 showed significant substrate hydrolysis in the presence of Zn^{2+}. There is no catalysis seen when Zn^{2+} is omitted from the reaction assay, and IgG 84A3 alone does not accelerate the rate of hydrolysis of **2**. *Thus the observed rate acceleration requires the presence of both antibody 84A3 and* Zn^{2+}. The antibody-catalyzed reaction depends on the type of metal ion used, as Cd^{2+}, Co^{2+}, or Ni^{2+} did not accelerate the rate of hydrolysis of **2** in the presence of antibody 84A3. The catalysis is highly specific for molecules congruent to the hapten in that **3**, **4**, and even the highly activated **5** (Figure 1) were not substrates for 84A3 with or without metals. Furthermore, catalysis is inhibited by the addition of **1b**.

Initial rates for the antibody-catalyzed hydrolysis of **2** in the presence of Zn^{2+} is shown in Figure 2, (k_{cat} = 2.7 min^{-1}). The uncatalyzed background rate of **2** (k_{uncat} = 0.00021 min^{-1}) and the metal ion-catalyzed background rate (**2** + Zn^{2+}, k_{uncat} = 0.0022 min^{-1}, Figure 3) were also determined. The affinity of 84A3 for both Zn^{2+} (data not shown) and **2**·Zn^{2+} ($K_{2\cdot Zn^{2+}}$ = 840 μM) was weak, while **2** was strongly bound to the antibody (K_2 = 3.5 μM). One minimal mechanism consistent with the data is shown in eq

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† A. P. Sloan Fellow (1993–1995).

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(6) All new compounds exhibited satisfactory spectroscopic (NMR, IR) and combustion analysis (±0.3%).

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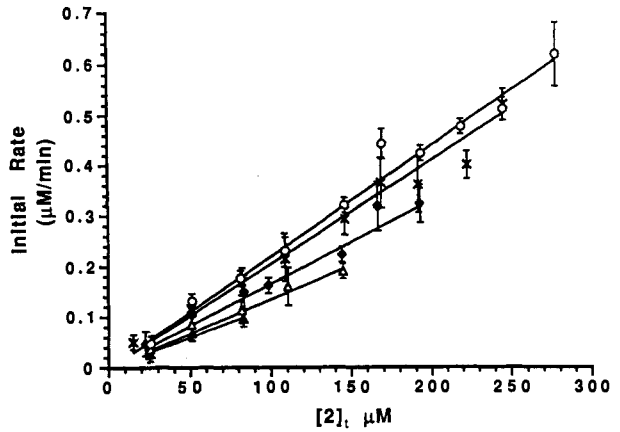
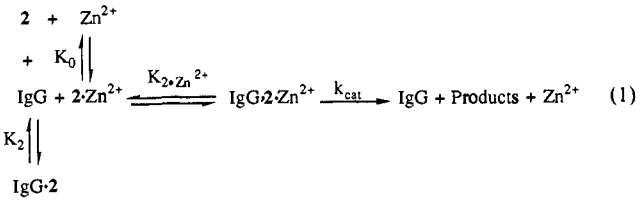


Figure 3. Initial rates of Zn-catalyzed background reaction. Error bars represent the standard deviation of at least two experiments. Data points: (from top) ○ 300 μM Zn, × 250 μM Zn, ◆ 200 μM Zn, △ 150 μM Zn, ▲ 100 μM Zn.

1.[7] Here the **2**·Zn^{2+} complex is the true substrate, with free **2**



inhibiting the reaction through the binary complex IgG·**2** (eq 1). An alternative to this mechanism, where a dead-end complex would be formed between antibody and zinc, is also possible; however, to fit the initial rate data equivalently the binding constant of the IgG 84A3·Zn^{2+} complex must be greater than 800 μM.

The antibody-catalyzed reaction is >1000 times the metal-catalyzed rate and >10 000 times the uncatalyzed rate. While such rate acceleration is respectable, this antibody-catalyzed reaction could be improved either by increasing the affinity of 84A3 for the **2**·Zn^{2+} complex or by decreasing the antibody's affinity for **2**. Such affinity attenuation may be achievable through new substrate design[8] or site-directed mutagenesis.

Previously we demonstrated that it was possible to use a "bait and switch" strategy to introduce catalytic groups in an antibody combining site. Here we show that this same strategy may be used to introduce metal cofactors in an antibody binding site. *Most remarkably is that a metal ion or a coordination complex need not be included within the hapten for the induction of antibodies that bind a metallo complex*[9] *and provide a suitable environment for catalysis.*[10]

Catalytic antibodies share many mechanistic features found in enzymes, including catalysis by transition-state stabilization, proximity effects, catalytic groups, and the use of cofactors. A method in which catalytic antibody efficiency may be increased would be to introduce multiple combinations of these enzymatic traits in the antibody combining site and have them all working in a concerted manner. The results we have presented are a step toward achieving such goals.

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Supplementary Material Available: Listing of synthetic, kinetic, and data analysis procedures; model curve fitting for eq 1; graphs for antibody-catalyzed and uncatalyzed reactions (8 pages). Ordering information is given on any current masthead page.

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