An Efficient Sol−Gel Reactor for Antibody-Catalyzed Transformations

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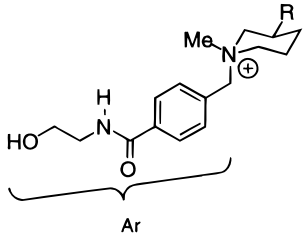
Since its inception, a decade ago, the science of catalytic antibodies has undergone a rapid maturation process, progressing from initial "proof of concept" and demonstration of fundamental enzyme-like characteristics to the catalysis of reactions that have no apparent biological counterparts. Catalytic antibodies, induced by immunization with stable transition-state analogues, have been shown to catalyze a remarkably broad scope of organic transformations, including difficult and unfavorable chemical reactions.[1]

One obvious need in antibody catalysis, particularly regarding their practical applications in organic synthesis, is to increase their cost-effectiveness. Much work has been done along these lines, including overexpression in bacteria, plant, seeds, and algae, as well as immobilization to facilitate the recovery of the catalyst. Covalent immobilization of catalytic antibodies onto solid supports[2] has already been achieved by Janda and co-workers,[2a] who have shown that the immobilized catalysts retain the same activity and stereospecificity as they exhibit in solution. Antibodies covalently immobilized onto glass beads, polystyrene, polycellulose, etc., may be packed in columns and used in continuous-flow reactors, but their effective concentration is quite limited.

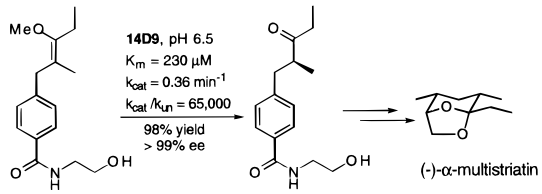
Sol−gel bioactive materials obtained by entrapment of proteins,[3] including purified enzymes,[4] whole-cell extracts,[5] and antibodies,[6] have attracted much attention.[7,8] Interest in these materials has stemmed from several of their unique properties, including synthetic simplicity of the immobilization procedure (which does not require covalent bonding to the protein) carried out under mild conditions, controllability of surface properties, enhanced thermal and pH stability, effective prevention of leaching of the entrapped proteins, convenient storage and recyclability for repeated use, and their ability to hold high matrix loads which are close to the concentration of the parent solution.[3−8] Following these potential advantages, we attempted to apply this methodology to catalytic antibodies. Here we report on the first successful entrapment of catalytic antibodies in a sol−gel matrix, and on their use in either a batch-wise operation or in a continuous flow apparatus. The flow reactor demonstrates the significance of this approach for preparative scale organic synthesis.

Antibody 14D9, which was elicited against hapten **1**, was selected for our initial immobilization studies. This



1 : R = $CH_2NHCO(CH_2)_3COOH$

antibody has already been proven an effective catalyst for various hydrolytic reactions, including the hydrolysis of a cyclic acetal,[9] ketals,[10,11] epoxides,[12] and enol ethers.[13,14] The latter reaction, which proceeds with high enantioselectivity, was used to demonstrate the advantages of catalytic antibodies in organic synthesis. For example, this reaction, while recycling the antibody with dialysis bags, produced gram-scale quantities of an enantiomerically pure ketone, **3**.[15] Moreover, this reaction represents the key step in the first total synthesis of a natural product using antibody catalysis.[16] It is noteworthy that all four asymmetric centers in the final product, (−)-α-multistriatin, originate from the chirality achieved in the antibody catalyzed step (eq 1).



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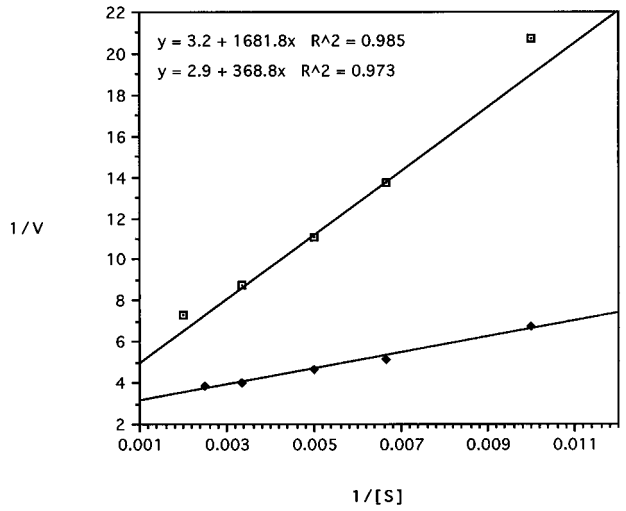


Figure 1. Lineweaver−Burk analysis of the antibody 14D9-catalyzed hydrolysis of **2** at pH 7.4 in PBS solution (circles) and within the sol−gel matrix (squares). Substrate concentration, $S = \mu$M; reaction rate, $v = \text{min}^{-1}$.

The entrapment of antibody 14D9 was carried out by mixing a phosphate-buffer saline (PBS) solution (50 mM phosphate, 100 mM NaCl, pH 7.4) of the mentioned antibody (0.045 mL, 2 mg/mL) with 0.005 mL of tetramethoxysilane (TMOS, Aldrich, 99%), followed by aging of the resultant gel for 24 h at room temperature. As already shown,[17] alcohol is an unnecessary additive in the silicon alkoxide sol−gel process, since it is released during the hydrolysis stage. Perfectly homogeneous matrixes are thus obtained. Buffered solutions of **2** (0.05 mL) at different concentrations (0.1−0.5 mM) were either placed over the porous gel matrix or mixed with a control solution of the antibody at the same concentration. The reaction was monitored with HPLC and the kinetic parameters at room temperature were obtained from the Lineweaver−Burk analysis of the HPLC data (Figure 1 and Table 1). Remarkably, we found that the entrapped antibody does not leach out, and yet, it is accessible to the substrate molecules.

It is evident from the Lineweaver−Burk plots (Figure 1) that catalysis follows classical Michaelis−Menten (MM) kinetics within the gel matrix (as is the case in solution). This is not a trivial result; it points out the fact that the entrapment yields a homogeneous population of dopants. In the case of alkaline phosphatase, for instance, a non-MM behavior was observed.[18] All kinetic data show that the turnover numbers (k_{cat}) are essentially independent of the media, suggesting that the catalytic step is not affected by the gel and that the substrate diffusion rate is much faster than the hydrolytic step. The slight increase in K_m observed in the reactions carried out with entrapped antibody could reflect hindrance of reorientational motions near the active site. The observed background reaction rate (k_{un}) is 2 orders of magnitude higher in the sol−gel matrix than in solution. This could reflect general acid catalysis by the silanol groups on the gel's surface. Indeed, a change of the buffer (Bis−Tris instead of PBS) did not result in any significant change of the background reaction rate. To verify this assumption we turned to another antibody-catalyzed reaction which is insensitive to general acid catalysis.

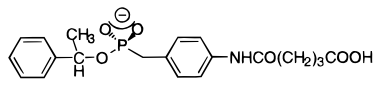
Table 1. Kinetic Parameters for Antibody 14D9-Catalyzed Hydrolysis of 2

medium	k_{cat} (min^{-1})	k_m (μM)	k_{un} (min^{-1})	k_{cat}/k_{un}
solution	0.073	140	1.70×10^{-6}	42 940
sol−gel matrix	0.065	520	1.33×10^{-4}	487

Table 2. Kinetic Parameters for Antibody 2H6-Catalyzed Hydrolysis of 5

medium	k_{cat} (min^{-1})	k_m (μM)	k_{un} (min^{-1})	k_{cat}/k_{un}
solution	0.47	4315	6.2×10^{-6}	76 000
sol−gel matrix	0.13	1467	6.2×10^{-6}	21 000

We selected antibody 2H6 which was elicited against hapten **4** and is known to catalyze the hydrolysis of inactivated esters, such as **5** (eq 2) under basic conditions.[19]



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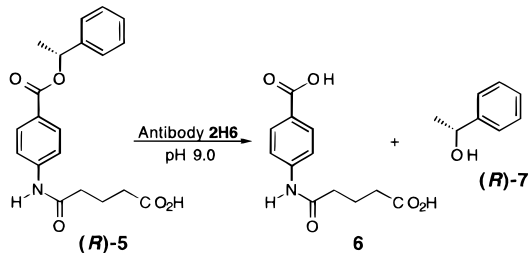

(2)

Two mixtures comprising **5** (100 mM) in Aces−Tris−Ethanolamine (ATE) buffer (50 mM, pH 9.0) were prepared: one was placed over a sol−gel matrix, prepared as described before, containing no catalyst, and the second was kept without gel, both at 24 °C for a period of 20 h. HPLC analysis of both samples indicated that the background reaction rate is unaffected by the gel ($k_{un} = 6.2 \times 10^{-6}\ \text{min}^{-1}$). When the reaction was carried out in the presence of the entrapped 2H6 (0.05 mg/mL) the rate enhancement (k_{cat}/k_{un}) was found to be 21 000 as compared with 76 000 observed when the same amounts of antibody were employed in homogeneous solution (Table 2). The MM behavior, observed also in this case, is perhaps the best indication for the homogeneity of the system.

To explore the applicability of the sol−gel entrapment method for preparative scale synthesis, we constructed a flow system apparatus. A 2H6 antibody solution in ATE (pH 9) was vigorously mixed with TMOS (90:10 v/v), forming a clear solution which was cast directly

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into an adjustable glass column (Western Analytical), equipped with a peristaltic pump and an accurate flow rate controller. The gel was allowed to age for 2 h and then flushed with a constant flow of buffer solution (1 mL/h) for 24 h. A solution of substrate **5** (100 μM; the substrate reservoir was kept at 0 °C in a water/ice bath) was added to the column and the progress of the hydrolysis reaction was monitored by HPLC.[20] The level of the background product under the experimental conditions was below 1%. After an initial drop during the first 9 h following the loading, the flow reactor reached steady state and was kept running at that level for an additional 65 h.[21] At this point the response of the reactor to a number of changes in conditions was tested. First, the buffer of the reservoir solution was replaced by Bis−Tris (pH 7). As expected, this pH change resulted in the complete cessation in the catalyzed product formation. The system responded well, however, when the original buffer was reestablished, and complete recovery of the antibody activity was achieved (Figure 2). Then the effect of added organic solvent and the recovery were tested. Thus, a solution of **5** in ATE (pH 9) containing 10% of CH_3CN was fed to the reactor causing an 80% drop of the steady-state productivity. Once again, the catalytic activity of the reactor was recovered when the substrate was eluted in the ATE buffer (Figure 2). We assume that the initial drop in activity may reflect blockage of the ultranarrow pores by the adsorption of the dicarboxylic product **6** on the silanol-rich surface of the matrix, leaving accessible and active the wider pores.[22]

In conclusion, we have shown here that the direct entrapment of proteins within ceramic matrixes is applicable to antibody catalysis. We have also demonstrated the advantages of using gel-entrapped antibodies in a continuous flow reactor. Diffusion rates of substrates and products within the gel matrix do not inhibit the catalytic reaction to any significant extent. Although this method appears to be general with respect to the reaction type, it is somewhat limited by the general acidity of the gel. Thus, the reaction conditions as well as the matrix properties will have to be optimized for any given reaction in order to maximize the rate enhancement of the catalyzed reaction, i.e., k_{cat}/k_{un}. The

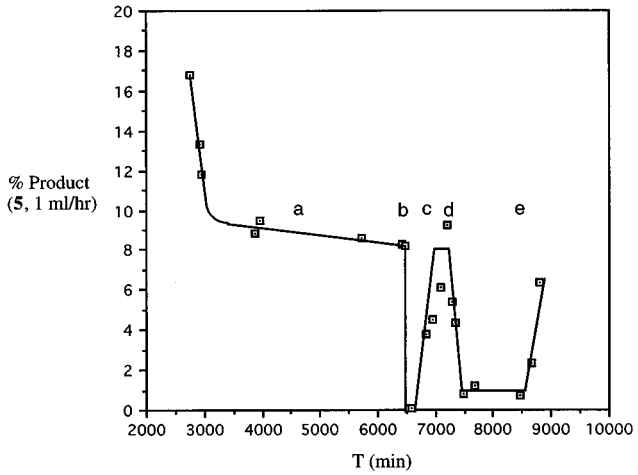

Figure 2. Response of the catalyzed hydrolysis of **5** by sol−gel entrapped 2H6 in a continuos flow reactor to variation in the reaction conditions (see text for explanations). (a) ATE pH 9, (b) Bis−Tris pH 7, (c) recovery with ATE pH 9, (d) ATE pH 9 + 10% CH_3CN, and (e) recovery with ATE pH 9. The flow rate was kept between 0.6 and 1.2 mL/h, with all measured conversions being normalized to 1 mL/h.

fact that catalytic activity within the gel matrix is conserved for long periods of time and withstands solvent changes suggests that this approach would be the method of choice for preparative scale organic synthesis. Taking advantage of the UV−vis transparency of the silica sol−gel matrixes, these biomaterials may also be used for photochemical reactions and for direct spectroscopic monitoring of the catalytic reactions. Work along these lines is currently underway.

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(20) All reactions were carried out at 24 °C and monitored at 254 nm by RP-HPLC (Hitachi L-6200A equipped with a Spherisorb column (25 cm × 4.6 mm, C18, 5 μm) using a mixture of two solutions: (A) acetonitrile:water (3:2) (B) water:trifluoroacetic acid (99.5:0.5) at 0.8 mL/min. The A:B ratio varied between 40:60 and 95:5 throughout the analysis.

(21) After periods of 24 h the gradual sealing of the sinter glass filter on the column could be perceived on the decrease of the flow rate. This problem could be easily overcame by the homogeneous blending of the gel within the column and the change in the flow direction when necessary. After proper reequilibration, these physical interventions had no influence on the catalytic behavior of the system.

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