Asymmetric Induction via a Catalytic Antibody[1]

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Recently it was shown that racemic phosphonate haptens can induce catalytic antibodies with either *R* or *S* substrate specificity, thereby accomplishing kinetic resolution.[3] As the next step, we chose to challenge a catalytic antibody with the possibility of enantioselective perturbation of a meso substrate. It was of particular interest to direct antibody-induced lipase-like action toward meso compound **1** (Figure 1).[4] It will be recalled that the corresponding *S* alcohol **2**, previously generated from the action of the electric eel acetylcholinesterase on **1**,[5] serves as a starting material for a concise stereospecific total synthesis of prostaglandin $F_{2\alpha}$[6] and congeners thereof (Figure 1).[7]

In undertaking this application, we would be implementing significant extensions of catalytic antibody methodology.[8] First we would be operating without any anchoring aryl substituents in the hapten **3** (Scheme I). Decreased immunogenicity was thus anticipated.[9] Also, the screening protocols would not benefit from a ready spectrophotometric assay.

We sought to apply compound **3** as a transition-state model. Its synthesis (Scheme I) in enantiomerically pure form posed an interesting question of feasibility. There had been concern that the deprotonation of **2** would be accompanied by racemization resulting from acyl migration.[10] In the event, under the conditions that were developed (Scheme I), no racemization occurred as assayed via ^{19}F NMR of the crude Mosher ester **4**.

Phosphonate **3** was coupled to carrier proteins BSA (bovine serum albumin) and KLH (keyhole limpet hemocyanin) via EDC (1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride) coupling methodology. Mice were immunized with the KLH conjugate of **3**, and antibodies were generated by standard protocols.[11] Immunization of **3** produced 33 antibodies of the IgG class. The abundance of acetyl- and butyrylcholinesterases in red blood cells and serum[12] necessitated extra caution during antibody purification. Ascites fluid was purified via anion-exchange chromatography (DEAE) followed by affinity chromatography (protein G) and then again by anion-exchange chromatography (Mono Q, Pharmacia). As a control, authentic acetyl- and butyrylcholinesterases were shown *not* to be retained on our affinity column under the conditions employed for antibody purification. Antibodies were judged to be homogeneous (>98%) by sodium dodecyl sulfate polyacrylamide gel electrophoresis.[13]

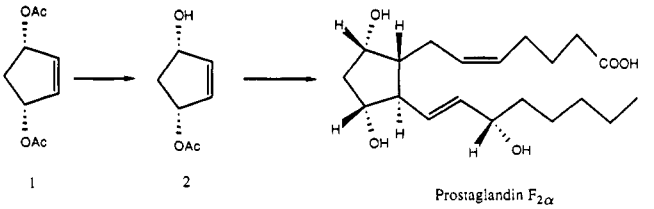
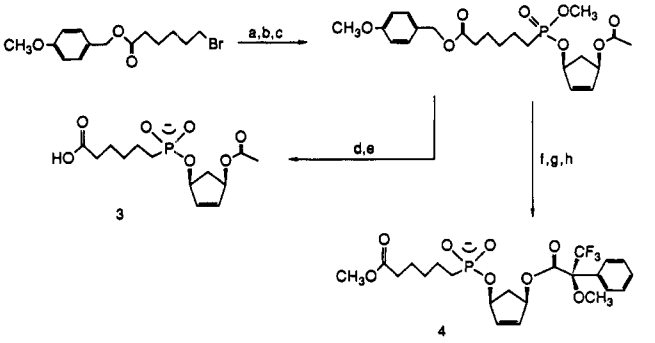


Figure 1. Meso substrate **1**; **2**, product from abzyme or enzyme reaction described and starting material for the synthesis of prostaglandin $F_{2\alpha}$.

Scheme I[a]



[a] (a) $P(OCH_3)_3$, *p*-cymene, 170 °C, 17 h, 62%. (b) $(COCl)_2$, CH_2Cl_2, room temperature, 6 h, 100%. (c) (1*R*,4*S*)-(+)-4-Hydroxy-2-cyclopentenyl acetate, LDA, THF, -78 to 0 °C, 2 h, 55%. (d) TFA, CH_2Cl_2, room temperature, 1 h, 90%. (e) *tert*-Butylamine, 50 °C, 2 weeks, 60%. (f) NaOMe, MeOH, 0 °C, 3 h, 100%. (g) (*R*)-(+)-α-(Trifluoromethyl)phenylacetyl chloride, pyridine, CH_2Cl_2, -30 °C to room temperature, 3 h, 85%. (h) *tert*-Butylamine, 50 °C, 2 weeks, 44%.

Table I. Kinetic Parameters for the Hydrolysis of *cis*-3,5-Diacetoxycyclopent-1-ene (**1**) to (1*R*,4*S*)-(+)-4-Hydroxy-2-cyclopentenyl Acetate (**2**)[a]

antibody/enzyme	K_m, 10^{-6} M	K_{cat}, min^{-1}	K_i,[e] 10^{-6} M
37E8[b]	177	0.007	7.0[f]
EC 3.1.1.7 (acetyl)[c]	620	250	630
EC 3.1.1.8 (butyryl)[d]	830	7.0	800

[a] Conditions: [b] Assay conditions (pH 8.0, ATE, [0.1 M *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES), 0.052 M Tris, 0.052 M ethanolamine], 10 μM antibody, 37 °C; under these conditions background hydrolytic rate 8×10^{-5} min^{-1}. [c] Type V-S, electric eel, Sigma, 1000–2000 units/mg of protein, used 0.059 units. [d] From horse serum, Sigma, 500–1000 units/mg of protein, used 0.0117 units. All assays consisted of determining **2** by GC analysis (Hewlett-Packard 5890 A, Carbowax 30m, 0.53-mm bore, capillary column) with ethyl levulinate as an internal standard. [e] Inhibition data determined with **3** as the inhibitor. [f] Determined by $S = K_m$, $K_i = I$, with velocity equivalent to $^1/_3V_{max}$.

Antibody-mediated production of **2** was initially screened by using 20 μM antibody and 1 mM diacetate **1** (Table I). One of the 33 antibodies, 37E8, was found to be catalytic. The initial rate of hydrolysis of **1** catalyzed by 37E8 (10 μM) followed Michaelis–Menten kinetics (Table I). The antigen **3** was a potent, tight-binding inhibitor of the reaction (Table I). Tight-binding inhibition, common among enzymes,[14] had previously been observed in an abzyme system.[15]

Our conclusion that the antibody is "contaminase" free is based not only on electrophoresis results but also on the large disparity seen betwen the abzyme and enzyme K_i's (Table I). If a cholinesterase were present in the antibody solution under the ex-

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perimental conditions employed,[16] our data (Table I) would dictate virtually no inhibition. The finding, where *extensive inhibition* was observed (greater than 60%), further supports the proposition that the lipase-like activity of the "abzyme" is not a consequence of contamination. In addition, the antibody (20 μM) shows minimal inactivation (less than 20% when **2** is 400 μM) in the presence of the powerful acetylcholinesterase inhibitor diisopropyl phosphorofluoridate (DFP) (50 μM).[17]

Enantioselectivity of the antibody-catalyzed reaction was investigated by GC analysis of a large-scale reaction of 37E8 (40 μM) in ATE (ACES, Tris, ethanolamine), pH 8.0, containing 200 μM **1**. At selected time intervals an aliquot of the reaction mixture was removed (600 μL), extracted twice with a 50/50 mixture of ethyl ether/ethyl acetate, and injected onto a microcapillary gas chromatographic column (Chrompack, CP-(optically pure)-cyclodextran-B-236-M-19). At 8 h, 60 μM **2** was detected with enantioselectivity in excess of 86% for the (1*R*,4*S*)-(+)-4-hydroxy-2-cyclopentenyl acetate (**2**). After 14 h, 100 μM **2** with an ee of 84% was detected. These results, while gratifying, appear to be limited only by the catalytic activity of abzyme 37E8 rather than by an inherent lack of enantiotopic group differentiation.

A comparison of abzyme 37E8 to acetyl- and butyrylcholinesterase (Table I) provided us with a crude index for an abzyme–enzyme comparison. Moreover, it provides us with alternative methods to check antibody purity. A comparison of k_{cat} (Table I) shows abzyme 37E8 to be some 3–4 orders of magnitude less potent catalytically than the cholinesterases. However, the inherent enantio group selectivity of 37E8 is excellent (>98% ee).[18]

The abzyme obtained here is admittedly somewhat primitive compared to its enzymatic counterparts. However, these findings suggest that asymmetric synthesis with catalytic antibodies could well provide attractive opportunities in organic synthesis. Further applications along these lines are envisioned.

(16) The following experiment was performed: Catalytic antibody 37E8 (10 μM) was added to a solution of 800 μM **1** and 25 μM **3**. These conditions were chosen so that the concentration of substrate would be at saturation for the antibody ($4.5K_m$) and approximately K_m for either cholinesterase.

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(18) Calculated values, which was approximated by correcting for the unwanted antipode (1*S*,4*R*)-(−)-4-hydroxy-2-cyclopentyl acetate, which came from the competing background hydrolytic reaction.

Quinone/Hydroxide Ion Induced Oxygenation of *p*-Benzoquinone to Rhodizonate Dianion ($C_6O_6^{2-}$) Accompanied by One-Electron Reduction to Semiquinone Radical Anion

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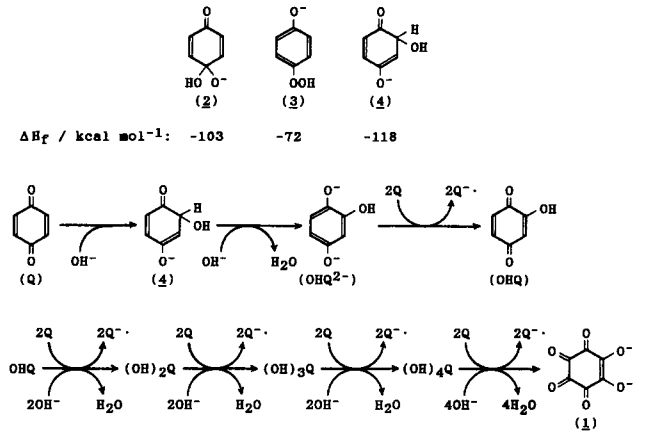
Hydroxide ion is regarded as a better electron donor as well as a stronger base in an aprotic solvent such as acetonitrile than in water, since the solvation energy for OH^- in an aprotic solvent is much less than in water.[1,2] Thus, OH^- in aprotic solvents has been reported to act as an electron donor toward quinones and other electron acceptors, since the radical anions of electron acceptors are often formed in the presence of OH^- in aprotic solvents.[2-6] The oxidized species of OH^- has been presumed to be H_2O_2[2-8] when no substitution reaction takes place.[9] However, no oxidized products of OH^- (H_2O_2 or O_2) have so far been definitely identified in the one-electron reduction of electron acceptors in the presence of OH^- in aprotic solvents, the mechanism of which remains to be solved.[2-6] This study reports that various quinones are readily reduced in the presence of $Me_4N^+OH^-$ to yield the corresponding semiquinone radical anions and that the one-electron reduction of *p*-benzoquinone (Q) to $Q^{\bullet-}$ is accompanied by the oxygenation of Q to rhodizonate dianion ($C_6O_6^{2-}$), which is the 10-electron-oxidized species of Q.

Upon mixing of *p*-benzoquinone (Q) with $Me_4N^+OH^-$ in deaerated MeCN at 298 K, Q is readily reduced to $Q^{\bullet-}$ (λ_{max} = 422 nm), and the yield based on the amount of OH^- is 100%.[10] The $Q^{\bullet-}$ thus formed is very stable in deaerated MeCN at 298 K. Similarly, the reactions of various quinones with $Me_4N^+OH^-$ yield the corresponding semiquinone radical anions.[11] No oxidized products of OH^- (H_2O_2 or O_2) have, however, been formed in the one-electron reduction of Q in the presence of OH^- in deaerated MeCN.[12] No oxidized products derived from the solvent (e.g., succinonitrile)[13] have been detected, either.[14] Then, the question

Scheme I



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(12) When the product mixture of the reaction of Q (2.0×10^{-2} M) with $Me_4N^+OH^-$ (2.0×10^{-2} M) was treated with Ph_3P (1.0×10^{-2} M) in deaerated MeCN, no formation of $Ph_3P{=}O$ was detected. When H_2O_2 was added to the same solution, however, Ph_3P was converted to $Ph_3P{=}O$ quantitatively in 40 min. Thus, it is concluded that no H_2O_2 is formed in the reaction of Q with OH^- in MeCN. The products in the gas phase were also analyzed by GLC using a molecular sieve 13 X column with He gas carrier. No appreciable formation of O_2 has been detected, either.

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