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단 신

키톤환원반응에 대한 항체촉매

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An Antibody-Catalyzed Reduction of Ketone

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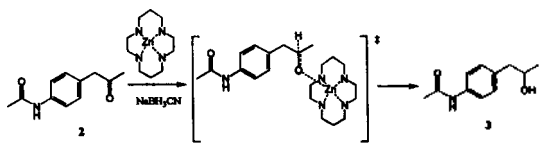
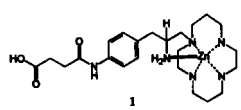
The development of enzyme-like catalysts with tailored specificity has been studied widely because it would increase the understanding on the principles of enzymatic catalysis and have many applications to biology, chemistry and medicine.[1]

Catalytic antibodies represent one promising approach to the development of enzyme-like catalysts.[2] Recently developed, hybridoma technology[3] has allowed the exploitation of the tremendous diversity of the immune system to generate monoclonal antibodies specific for virtually any molecule of interest. Consequently, the development of strategies for introducing catalytic activity into antibody binding sites has lead to a new class of enzyme-like catalysts. Based on principles of the known enzymatic catalysis,[4] several strategies[5] for catalytic antibodies have been described, which are included transition state stabilization effects, introduction of catalytic functional groups into antigen binding site using charge complematarity and π-stacking effect, and medium effects. Thus, antibodies were found to catalyze the various organic reactions from simple hydrolysis of ester to pericyclic reactions such as Diels-Alder reaction.[6] However, only a few catalytic antibodies using cofactors to catalyze the target reactions have been described in spite that cofactors in enzymatic catalysis were widely recognized. Particularly, catalytic antibodies containing catalytically important metal ions such as zinc, cobalt, copper and nickel were unexplored in spite of the important roles of metal ions in enzymatic catalysis.[7]

Here, to develope metallo-catalytic antibodies and thus expand the scope of antibody catalysis, a zinc-containing antibody which found to catalyze $NaBH_3CN$-reduction of ketone is described. Particularly, reduction of ketone is one of the most important organic reactions in synthesis of natural products and pharmaceuticals.[8] Although enzymes and small organic catalysts are being used widely for the selective reduction of ketones, their uses are quite limited in the terms of reaction condition, substrate compatability and selectivities. Thus catalytic antibodies may provide an alternate way to develop novel catalysts useful for organic synthesis.

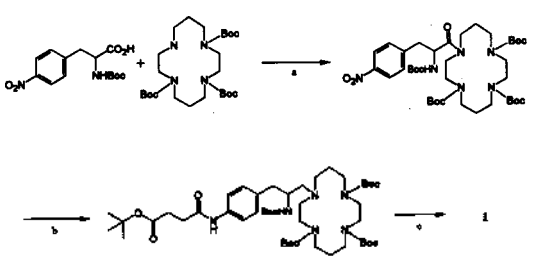




RESULTS ANS DISCUSSION

To elicit that catalytic antibody, hapten **1** was designed. In **1**, metal ion can complex with nitrogens in cyclam moiety and lariat amine linked through one nitrogen of cyclam. Thus antibodies which are generated against **1** have metal binding site with the close proximity to the carbonyl group of ketone substrate. As a result, metal ion can act as a Lewis acid to polarize the carbonyl group of ketone substrate and thus catalyze the reduction of ketone by electrophilic catalysis mechanism. Furthermore, amine-attached carbon of **1** might mimics the sp^3-like carbon of the proposed transition state of reduction of ketone.[9] Therefore, the antigen binding sites of the corresponding antibodies would stabilize selectively the transition state of reduction of ketone and thus the corresponding antibodies may catalyze the target reaction.

As shown in *Scheme*, hapten **1** was synthesized by following the standard procedures using cyclam as starting material. Hapten **1** was coupled to keyhole limpet hemocyanin (KLH) using the standard EDC-promoted amide formation reaction between carboxylic acid of **1** and δ-amine of surface lysine of KLH. The formation of KLH-hapten conjugate was confirmed by the UV/VIS spectroscopy and epitope density was 12.5. The resulting KLH-hapten conjugate was used to immunize Balb/c mice. By using standard hybridoma technology,[10] 23 monoclonal antibody specific to the hapten **1** were generated. The conversion of ketone **2** to alcohol product **3** in aqueous 5 mM NaCl, 50 mM MES buffer, 1 mM cyclam, 1 mM Zn^{2+}, 50 mM $NaBH_3CN$, pH=5.5, was followed by HPLC.



Scheme 1. Synthesis of **1**: (a) DCC, HOBT. (b) i. Borane-THF, ii. H_2/Pd-C, iii. mono-tBu-succinate, DCC/HOBT. (c) i. 6N HCl, ii. $ZnCl_2$.

One of 23 antibodies, 4B6.1, was found to catalyze $NaBH_3CN$-reduction of ketone in the presence of zinc over the background reaction, with initial rate consistent with Michaelis-Menten kinetics. The value of k_{cat} and K_m were determined by fitting the kinetic data at the various concenturations of substrate **2** to the Michaelis-Menten equation ($\upsilon_i = k_{cat}[E]_{total}[S]/\{[S]+K_m\}$) and are 7.48×10^{-5} m^{-1} and 334 M, respectively, at 37 °C. Thus the second order rate constant (k_{cat}/K_m) was 0.224 $m^{-1}M^{-1}$. The second order rate constant for the uncatalyzed reaction under the same condition was 1.30×10^{3} $m^{-1}M^{-1}$, corresponding to a rate enhancement of roughly the order of 10^2 over the uncatalyzed reaction. Under the same condition without cyclam or zinc ion, antibody 4B6.1 did not accelerate the reaction. Moreover, cyclam-Zn complex itself without antibody 4B6.1 was found to catalyze the reaction weakly (about 5 times). Thus zinc ion in antibody binding site has an important role in catalysis. Also, this antibody 4B6.1 did not catalyzed reaction when zinc ion was replaced with other metal ions such as nickel, copper, cobalt, indicating that the antibody-catalyzed reaction is selective as shown in many metalloenzyme catalysis. Surprisingly, this antibody-catalyzed reaction was not inhibited by hapten. This may imply that antibody-catalyzed reaction did not occur in antibody binding site or binding strength of antibody-antigen is not large enough to inhibit the binding of substrate with antibody. Although the exact nature of this antibody catalysis is not clear, it is obvious that antibody 4B6.1 catalyze the reduction of ketone **2**. Additional mechanistic[11] and structural studies on antibody 4B6.1 may clarify the exact nature of catalysis.

In summary, a zinc binding antibody which accelerates the rate of $NaBH_3CN$-reduction of ketone was successfully elicited against hapten **1** in spite that the reaction mechanims is not clear.[12] This study show that many metal ion dependent reactions

Table 1. Kinetic data of I g 4B6.1 catalyzed reduction of **2**

[2], μM	100	175	250	500	1000
10^{10} υ_i, M/min	1.75	2.55	3.19	4.52	5.60

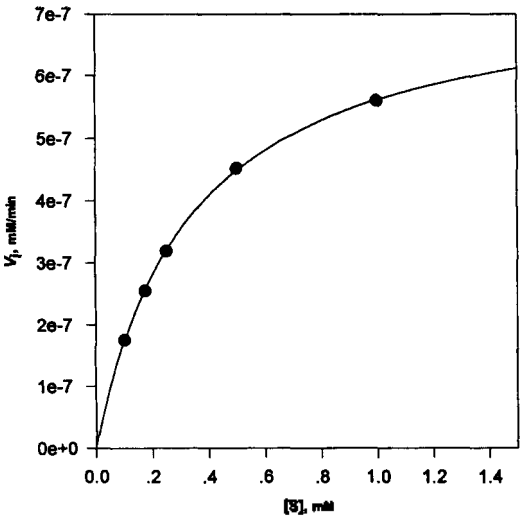


Fig. 1. V_i vs. [S] plot for 1 g 4B6.1 catalyzed reduction of **2**.

can be catalyzed by antibodies generated against the carefully designed haptens.[13] Also, this study suggests that catalytic effect of cofactors could lead to large increase of catalytic power of antibodies when combined with the known effects of catalysis.

EXPERIMENTAL

Synthesis of 1. To a solution of 0.7 g of Tri-Boc-cyclam-(N-Boc-p-nitrophenylalanine) amide (0.89 mmol)[14] in 30 mL of THF was added 10 mL of 1M Borane-THF complex in THF (10 mmol). After stirring overnight at r.t. and reflux for 2 hr, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 5% MeOH in CH_2Cl_2 to give an amorphous white solid (0.265 g, 38%).

The resulting nitro compound (263 mg) was dissolved in 10 mL of methanol and Pd-C (200 mg) was added. The reaction mixture was stirred overnight at r.t. under hydrogen atmostphere. After filtering off the catalysts, all volatiles were removed at reduced pressure. To a solution of the resulting amine and 70 mg of mono-tBu succinate in 10 mL of THF was aded 35 mg of HOBT and 84 mg of DCC. After stirring overnight at r.t., all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 5% MeOH in CH_2Cl_2 to give an amorphous white solid (0.30 g, 97%): ^{1}H NMR ($CDCl_3$/CD_3OD=1/1, 200 MHz) δ (ppm) 8.18 (d, J=9.5 Hz, 2H), 8.00 (d, J=9.5 Hz, 2H), 4.68 (q, J=7.5 Hz, 1H), 3.20 (m, 16H), 2.95 (d, J=7.5 Hz, 2H), 2.22 (t, J=7.2 Hz, 2H), 2.12 (t, J=7.2 Hz, 2H), 1.43 (s, 27H) 1.32 (s, 9H), 1.22 (s, 9H); ^{13}C NMR ($CDCl_3$/CD_3OD=1/1, 300 MHz) δ (ppm) 175.5, 171.4, 169.8, 168.5, 166.9, 139.6, 135.6, 128.1, 120.3I, 68.3, 67.2, 67.0, 56.2, 55.6, 54.9, 54.3, 49.7, 48.1, 47.9, 47.1, 46.5, 38.5, 36.7, 31.3, 30.8, 30.1 29.5, 21.5, 20.7; IR (neat) 3321, 2874, 1722, 1674, 1573 cm^{-1}; MS (FAB) *m/z* 906 (M+1).

To a solution of product of the above reaction in 10 mL of MeOH was added 1 mL of 6 N HCl solution. After stirring overnight at r.t., all volatiles were removed at reduced pressure. The resulting HCl salts were dissolved in 1 mL of PBS buffer and 1 equiv. of $ZnCl_2$ was added. The formation of Zn^{2+} complex was confirmed by the UV/VIS spectroscopy and Zn^{2+} complex was used for immunization without the further purifications.

Kinetic measurement. Initial rates were determined at five different substrates concenturations (100 μM, 175 μM, 250 μM, 500 μM, 1000 μM) by HPLC measurements of product formation with 5 time intevals at less than 10% completion of reaction. HPLC assays using Rainin S-3200 HPLC were carried out with a Microsorb C18 reverse-phase column with a gradient starting 10% acetonitrile in water and increasing to 100% acetonitrile over 25 min. Products formation was monitored at 270 nm and quantitated against the internal standard benzoic acid. Antibody concentrations were 10.0 μM in binding sites.

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11. The rate dependence on pH could not be measured because of the deactivation of $NaBH_3CN$ above pH =7 as well as the precifitation of antibodies below pH=5.
12. *Other examples for reduction of ketones by antibodies*: (a) Suh, J.; Oh, E.; Kim, S.; Lee, C. S.; Jeong, G. *Bull. Kor. Chem. Soc.* **1991**, *12*, 352. (b) Nakayama, G. R.; Schultz, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 780. (c) Heish, L. C.; Yonkovich, S.; Kochersperger, L.; Schultz, P. G. *Science*, **1993**, *260*, 337.
13. Other reactions such as reductive amination, cyanohydrin formation and Strecker reaction of ketone were also found to be catalyzed by antibody 4B6.1 but the rate accelerations are relatively small (the order of 10). But unfortunately, none of antibody was found to catalyze metal ion assisted-hydrolysis of ester, acetal, phosphonic ester and amide.
14. Tri-Boc-cyclam-(N-Boc-p-nitrophenylalanine) amide was synthesized from the commercially available starting material, cyclam, by following the standard reactions.[15] A base (K_2CO_3) catalyzed monobenzylation of cyclam with benzylbromide followed by Boc protection with $(Boc)_2O$, hydrogenolysis of benzyl group and DCC coupling with N-Boc-p-nitrophenylalanine afforded Tri-Boc-cyclam-(N-Boc-p-nitrophenylalanine) amide.
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