at −5℃ was added a slight excess of $NaBH_4$ or $NaBH_3CN$. After it was stirred for 1 h, the solution was evaporated to dryness extracted with diethyl ether (100 ml). Evaporation of the ether gave a yellow crystalline solid of the ortho and meta adducts (80 % yield). The ratio of ortho- and meta-adduct was determined by comparing the integration of ^{1}H-NMR spectrum. The mixture has the following spectral properties: IR ν_{CO} 2015, 1920 cm^{-1}, $\nu_{C=O}$ 1655 cm^{-1}. Anal. Calcd. for $C_{22}H_{11}MnNO_4$: C, 63.93; H, 3.90; N, 3.39. Found: C, 63.11; H, 4.18; N, 3.49. Ortho-adduct: ^{1}H-NMR($CDCl_3$) δ 2.54(t, 6 Hz, H^5), 2.58(d, 13.7 Hz, $H^{6\text{-exo}}$), 3.04(dd, 13.7 Hz, 6 Hz, $H^{6\text{-endo}}$), 4.13(t, 6 Hz, H^4), 4.46(d, 6 Hz, H^2), 4.97(t, 6 Hz, H^3) 6.71−7.40(m, Ph) ppm. Meta-adduct: ^{1}H-NMR($CDCl_3$) δ 1.86(d, 11.6 Hz, $H^{6\text{-exo}}$), 2.32(m, $H^{1\text{ and }5}$), 3.05(ddd, 11.6 Hz, $H^{6\text{-endo}}$), 4.26(t, 6 Hz, H^4), 6.19(d, 6 Hz, H^3), 6.68-7.28(m, Ph) ppm.

5. (a) The reaction was studied by using a slight excess of $LiAlH_4$ in THF at 0℃. Ms, m/z, 218(M^+), 190(M^+-CO), 134(M^+-2CO). (b) G. Winkhaus and G. Wilkinson, *Proc. Chem. Soc.*, 31 (1960); G. Winkhaus, L. Pratt, and G. Wilkinson, *J. Chem. Soc.*, 3807 (1961).
6. A 52% isolated yield of ortho- and meta-adduct was obtained. The ratio of ortho- and meta-adduct was determined to be 75:25. The mixture has the following spectral properties: IR ν_{CO} 2000, 1920 cm^{-1}, $\nu_{C=O}$ 1650 cm^{-1}. Ms, m/z, 427(M^+), 399(M^+-CO), 371(M^+-2CO), 343(M^+-3CO), 287(M^+-$Mn(CO)_3$-H). Ortho-adduct: ^{1}H-NMR($CDCl_3$) δ 0.73 (d, 6.4 Hz, Me), 3.43-3.53(t+m, $H^{5\text{ and }6}$), 4.76(t, 5.7 Hz, H_4), 5.00(d, 5.8 Hz, H^2), 5.66(t, 5.8 Hz, H^3), 6.91-7.60 (m, Ph) ppm. Meta-adduct: ^{1}H-NMR($CDCl_3$), 0.50(d, 6.5 Hz, Me), 2.64(m, H^6), 3.10(m, $H^{1\text{ and }5}$), 4.82(t, 5.6 Hz, H^4), 6.36(d, 5.6 Hz, H^3), 7.00-7.80(m, Ph) ppm.
7. The isomer ratio was determined by the integration of ^{1}H-NMR spectrum.
8. A 70% isolated yield of meta-adduct was obtained. IR ν_{CO} 2020, 1925 cm^{-1}, ν_{C-O} 1655 cm^{-1}, ν_{CN} 2230 cm^{-1}. Ms, m/z, 480(M^+), 452(M^+-CO), 424(M^+-2CO), 396(M^+-3CO), 340(M^+-$Mn(CO)_3$-H). ^{1}H-NMR($CDCl_3$) δ 1.05(d, 5.8 Hz, Me), 2.56(t, 5.9 Hz, H^5), 3.19(t, 5.9 Hz, H^6), 3.51(d, 5.7 Hz, H^1), 5.05(t, 6.2 Hz, H^4), 6.17(d, 6.2 Hz, H^3), 7.16-7.34(m, Ph) ppm.
9. A 50% isolated yield of ortho-adduct was obtained. IR ν_{CO} 2010, 1910 cm^{-1}, ν_{C-O} 1640 cm^{-1}, ν_{CN} 2240 cm^{-1}. ^{1}H-NMR(C_6D_6) δ 1.29(d, 7.7 Hz, CH_2), 2.51(t, 6.4 Hz, H^5), 3.55(tt, 6.4 Hz, H^6), 3.96(t, 5.8 Hz, H^4), 4.60(d, 5.9 Hz, H^2), 4.70(t, 5.8 Hz, H^3), 6.80-7.50(m, Ph) ppm. Anal. Calcd. for $C_{23}H_{17}MnN_2O_4$: C, 63.73; H, 3.79; N, 6.19. Found: C, 63.69; H, 4.55; N, 5.45.
10. IR ν_{CO} 2107, 1920 cm^{-1}, ν_{C-O} 1710, 1655 cm^{-1}. Ms, m/z, 413(M^+-2CO), 385(M^+-3CO), 342(M^+-3CD-CH_3CO). Anal. Calcd. for $C_{25}H_{20}MnNO_5$: C, 63.97; H, 4.30; N, 2.98. Found: C, 64.65; H, 5.68; N, 2.87.
11. (a) Y. C. Chung, D. A. Sweigart, and P. G. Williard, *Organometallics*, **1**, 1053 (1982); (b) Y. K. Chung, H. K. Bae, and I. N. Jung, *Bull. Kor. Chem. Soc.*, **9**, 349 (1988); (c) T. -H. Hyeon, T. -M. Chung, and Y. K. Chung, *Bull. Kor. Chem. Soc.*, **10**, 500 (1989).
12. Y. -N. Lee, Y. K. Chung, Y. Kim, and J. H. Jeong, *Organometallics*, **9**, 2851 (1990).

Antibody for L-Mandelate. Kinetic Assay of Monoclonal Antibody and Optical Resolution with Antibody

Junghun Suh*, Eugene Oh, Soo Hyung Kim, Chang Sun Lee, and Gajin Jeong*,†

Department of Chemistry, and †Department of Microbiology, Seoul National University, Seoul 151-742

Received February 27, 1991

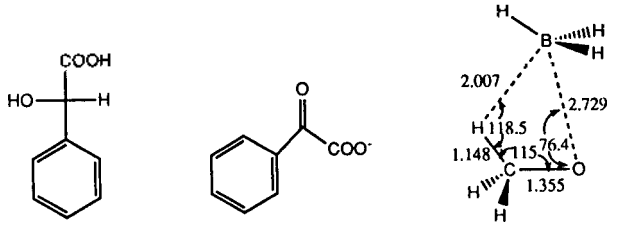
During the past few years, the area of catalytic antibodies has emerged as a new field of both chemistry and biology.[1-3] In principle, tailor-made artificial enzymes can be obtained by designing antibodies capable of stabilization of the transition state of the target reaction. At present, efforts are primarily made on development of basic concepts and techniques for the enhancement of the efficiency of catalytic antibodies.

The primary biological function of antibodies is recognition of the structure of the antigen and very strong complex formation with the antigen. In order to develop a catalytic antibody, induction of the proper structure of the binding site of the antibody is attempted by using antigens resembling the transition state of the target reaction. Since antibodies are not readily produced against an antigen with a small size, the transition-state analogue is linked as a hapten to a large molecule such as proteins. When the resultant antigen with a large size is used for production of the antibody, a great number of antibodies are formed, each recognizing a small portion of the antigen. Then, monoclonal antibodies with correct catalytic behavior are slected from the polyclonal antibodies. In the study of catalytic antibodies, the design of the structure of the transition-state analogues and the selection of the right monoclonal antibodies are among the most crucial steps.

Several assay techniques are known for the selection of the monoclonal antibodies from the mixture of antibodies formed in immunological response to a single antigen. These include solid-phase assays such as ELISA (Enzyme Linked Immuno Sorbent Assay) or IRMA (Immuno Radio-Metric Assay), soluble-phase assay, immunodouble diffusion, cellular assay, biological assay, and immunocytochemical assay.[4] Monoclonal antibodies recognizing the hapten can be selected by using these techniques. Even if a monoclonal antibody specific for the hapten is selected, the chance that the antibody recognizes the functional group introduced to the hapten as the transition state analogue and, furthermore, catalyzes the target molecule is not large.

We have been interested in the development of kinetic methods for the selection process. If the ability to catalyze the target reaction is tested in the selection process, monoclonal antibodies with the desired catalytic ability can be, in principle, chosen at this stage. Toward this end, we have tested the kinetic assay method in the preparation of the monoclonal antibodies for L-mandelate(**L-1**). Hapten **L-1** was coupled to γ-globulin, the carrier protein, at pH 5.0 and 4℃ by using 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide, followed by exhaustive dialysis against 10 mM phosphate, 150

mM NaCl buffer, pH 7.4.[5] Thus, the antigen is an L-mandelamide, as the carboxylate of L-mandelate is coupled with the primary amine of the carrier protein under this coupling condition. Balb/c mice were immunized with this antigen. Three days after final (the 4th boosting) injection, the spleen cells of an immunized mouse were fused with myeloma cells with polyethyleneglycol.[6] The fused cells were incubated at 37℃, and then monoclonal antibodies were selected from the resulting subclonal cells.



L-1 2 3

For the kinetic assay method in the selection process of the monoclonal antibodies, the reduction of benzoylformate (**2**) with $NaBH_4$ was used. An *ab initio* MO calculation for the $NaBH_4$-reduction of formaldehyde in the gas phase indicated a transition state (**3**) in which the hydride transfer is almost complete while the new B-O bond is not yet formed.[7] If a similar structure is assumed for the reduction of **2**, the transition state would resemble **1** very closely. Then, the antibody recognizing the mandelamide portion of the antigen might catalyze reduction of **2** with $NaBH_4$.

Selection of monoclonal antibody specific for L-**1** was made by assaying each of the 72 subclonal cells for the ability to promote the reduction of **2** with $NaBH_4$. In aqueous media, the hydrolysis of $NaBH_4$ occurs readily at low pHs, and complete hydrolysis of $NaBH_4$ terminates the reduction reaction. The pH, however, cannot be raised to a very high value simply to suppress the hydrolysis rate of $NaBH_4$ since antibodies are destabilized at high pHs. The conditions of the kinetic assay were chosen as 0.05 M Tris buffer, pH 8.02 and 25℃ in the presence of 14% (v/v) ethanol. Under these conditions, pH was maintained constant during the whole assay process. The reduction of **1** with $NaBH_4$ is accompanied by a large absorbance decrease (1.2 $cm^{-1}M^{-1}$ for 1×10^{-4} M **1**) at 250 nm. Under the assay conditions, $NaBH_4$ was completely hydrolyzed within 5 min, stopping the reduction of **1**. In the absence of any added antibodies, about 7% of the initially added **1** was reduced. From the magnitude of absorbance decrease observed in the presence of the added multi-well soup solution (100 μl) of each of the subcloned cells, the catalytic ability of the corresponding antibody was estimated. On the basis of this kinetic assay, subcloned cells JS-I-15 and JS-I-60 were selected and monoclonal antibodies were prepared by using these cells.

About 10^6 hybridoma cells of JS-I-15 or JS-I-60 were inoculated intraperitoneally into a mouse which had been primed with 0.5 ml pristane (2,6,10,14-tetramethylpentadecane). About 3-5 ml of ascitic fluid was usually trapped in the abdomen 10 to 14 days after inoculation. Immunoglobulin G was purified from ascites fluid by affinity chromatography on protein A coupled Sepharose 4B and dialyzed exhaustively against 0.05 M Tris buffer (pH 8.02).[8] The monoclonal antibodies thus obtained were judged to be homogeneous by

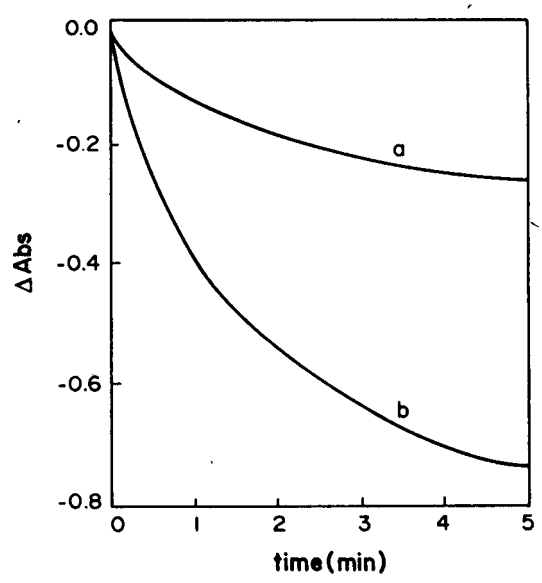


Figure 1. Absorbance (ΔAbs) changes (at 250 nm) accompanying the reduction of **2** (1×10^{-4} M) with $NaBH_4$ (1×10^{-3} M) in the absence (curve **a**) and the presence (curve **b**) of monoclonal antibody JS-I-60 (1×10^{-6} M) at pH 8.02 (0.05 M Tris) and 25℃ in the presence of 14% (v/v) ethanol.

10% sodium dodecyl sulfate-polyacrylamide-gel electrophoresis with Coomassie blue staining.[9]

Of the antibodies obtained from the two subcloned cells selected by the kinetic assay method, only monoclonal antibody JS-I-60 manifested satisfactory behavior as an antibody against L-**1**. In Figure 1, the absorbance changes observed at 250 nm for the reduction of **1** in the absence and the presence of monoclonal antibody JS-I-60 are illustrated. In the presence of 1×10^{-6} M antibody, the degree of rate acceleration is not very large. This is not unexpected since the antibody can be bound very strongly by L-**1**, the product of the reduction reaction, and the subsequent inhibition by the product can inactivate the catalytic activity of the antibody when the concentration of the product reaches to a sufficiently high level ($[I]>K_i$). That monoclonal antibody JS-I-60 recognizes the L-mandelate portion of the antigen is more affirmatively proved by resolution of DL-**1** with the antibody coupled to an HPLC column. The monoclonal antibody (2.5 mg) was coupled to a 5 cm×5 mm (i.d.) Selecti-Spher-10™ Activated Tresyl column (Pierce). This column was then used for resolution of DL-**1** dissolved in 0.1 M NaH_2PO_4, pH 7.4. When 10 μl DL-**1** was subjected to separation on the column containing the antibody, only half of the mandelate was eluted (detection at 250 nm). On the other hand, L-**1** was very tightly bound by the column and repetitive washing of the column with citrate buffer (0.1 M, pH 4.5 and 1.3) did not remove the residual L-**1**.

The monoclonal antibody prepared in this investigation does not manifest efficient catalytic activity toward the reduction of benzoylformate. Nevertheless, the results of the present study demonstrate that the kinetic assay method may be applied for the selection process of catalytic monoclonal antibodies. In the present study, selection of subcloned cells were performed only by the kinetic method. When the kinetic method is used in conjunction with other conventional

methods such as ELISA, the efficiency of the assay of monoclonal catalytic antibodies might be improved considerably. The resolution of DL-**1** with the antibody prepared in this study is not practical since the L-form is not released readily after complexation with the antibody. However, the present investigation clearly indicates that monoclonal antibodies can be utilized as reagents for optical resolution. The low efficiency in the catalysis of the reduction of benzoylformate and the lack of practical applicability of the resolution of DL-**1** is due to the very high affinity of the antibody toward L-**1**. In this regard, we are investigating different reactions in which the products less strongly bound by the monoclonal antibody.

Acknowledgment. This work was supported by a grant from the Korea Science and Engineering Foundation. E.O. was supported by a fellowship from Organic Chemistry Research Center/KOSEF.

References

1. P. G. Schultz, R. A. Lerner, and S. T. Benkovic, *C & EN*, May 28, 26-40 (1990).
2. P. G. Schultz, *Acc. Chem. Res.*, **22**, 287 (1989).
3. J. Suh and E. Oh, *Prog. Chem. Chem. Ind.* (Korean Chemical Society), **29**, 822 (1989).
4. A. M. Campbell, "Monoclonal Antibody Technology", Elsevier, Amsterdam, Chap. 2 (1984).
5. B. Erlanger, *Meth. Enzymol.*, **70**, 85 (1980).
6. A. Johnstone and R. Thorpe, "Immunochemistry in Practice", Blackwell Scientific Publications, Oxford (1982).
7. O. Eisenstein, H. B. Schlegel, and M. M. Kayser, *J. Org. Chem.*, **47**, 2886 (1982).
8. G. Kronvall, J. Grey, and R. Williams, *J. Immunol.*, **105**, 1116 (1970).
9. V. Laemmli, *Nature* (London), **227**, 680 (1970).