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A New Competitive Inhibitory Monoclonal Antibody to Carboxypeptidase A (CPA)

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Abstract: The monoclonal antibody $ID_{11}D_7$ to carboxypeptidase A (CPA) was prepared. The inhibitory effect of McAb $ID_{11}D_7$ on the peptidase activity of CPA was measured. The result reflects that the McAb $ID_{11}D_7$ can competitively inhibit the peptidase activity of CPA with an apparent inhibitory constant of 6.3×10^{-9}M. Based on this work, the McAb $ID_{11}D_7$ can be used as idiotypic antigen to prepare anti-idiotypic catalytic antibody with the peptidase activity similar to CPA.

Keywords: Monoclonal antibody, carboxypeptidase A (CPA), competitive inhibition, peptidase activity.

Catalytic antibodies are a new class of biocatalysts, and have been used to catalyze many types of chemical reaction successfully[1]. At present, it is difficult to use catalytic antibodies to catalyze the hydrolysis of amide bond. How to design and prepare the catalytic antibodies that can effectively catalyze the hydrolysis of amide bond has been an unsolved problem. But the hydrolysis and synthesis of amide bond are very important chemical and biological reactions. Therefore, to produce the catalytic antibodies which can catalyze the hydrolysis of amide bond is a significant research work..

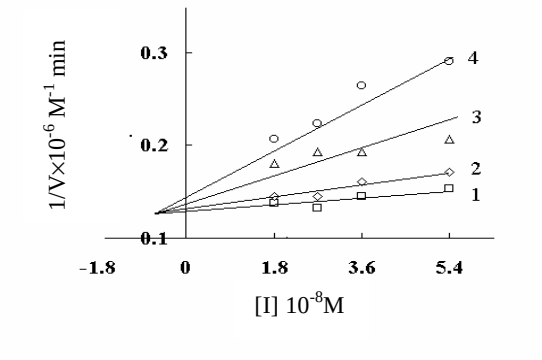
A method of preparing catalytic antibodies is to produce anti-idiotypic antibodies to enzyme in order to obtain the catalytic antibodies which may have similar activity to natural enzyme. A. Friboulet successfully produced the anti-idiotypic antibody to acetylcholinesterase. This anti-idiotypic antibody has the similar enzymatic activity to acetylcholinesterase[2]. Carboxypeptidase A (CPA) catalyzes the hydrolysis of the C-terminal amide bond[3]. Therefore, with carboxypeptidase A (CPA) to produce it's anti-diotypic antibody, we may obtain the catalytic antibody with the peptidase activity similar to CPA. The key step of this method is to produce the first Ab (Ab_1) which combines with the active sites of CPA and competitively inhibits the peptidase activity of CPA.

Balb/c mice were immunized with CPA. By means of cell fusion, ELISA assay, subcloning and the kinetic inhibition experiments, we obtained a monoclonal antibody $ID_{11}D_7$ which competitively inhibits the peptidase activity of CPA. The McAb $ID_{11}D_7$ was identified as IgG_1 subclass, binding constant to CPA is $9.8\times10^{8}M^{-1}$.

The inhibition experiment of the peptidase activity of CPA with McAb $ID_{11}D_7$ was performed. 2 μg CPA (5×10^{-8}M) was incubated with increasing concns. (1.8×10^{-8}—5.4

$\times 10^{-8}$M) of McAb $ID_{11}D_7$ for 1 hr at room temperature. Then the residual peptidase activity was measured in a substrate concentrations range of (0.4×10^{-3}M~1.0×10^{-3}M). A Dixon plot of the experimental data obtained is given in **Figuge 1**.

Figure 1. Dixon plot representing the inhibition of the rate of hydrolysis of hippuryl-L-phenylalanine (V) with CPA by McAb $ID_{11}D_7$ (I). Ki: inhibitory constant; Substrate concns. used: ① 1.0×10^{-3}M; ② 0.8×10^{-3}M; ③ 0.6×10^{-3}M; ④ 0.4×10^{-3}M



The result reflects in a low range of molar concentrations (1.8×10^{-8}~5.4×10^{-8}M) of McAb $ID_{11}D_7$, the McAb $ID_{11}D_7$ shows its inhibition of the peptidase activity of CPA, with an apparent inhibitory constant 6.3×10^{-9}M. That suggests the McAb $ID_{11}D_7$ combines with the active sites of CPA, and is good for being used as idiotypic antigen to produce the anti-idiotypic antibody which may have the peptidase activity similar to CPA.

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