Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1487–1492

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A New Monoclonal Anti-idiotypic Catalytic Antibody with a CPA-like Activity

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Received 25 January 1999; accepted 9 April 1999

Abstract: IIF_9D_8, a new monoclonal anti-idiotypic catalytic antibody with a CPA esterase-like activity was elicited by $ID_{11}D_7$, the monoclonal competitive inhibitory antibody to CPA. The hydrolysis of hippuryl-DL-phenyllactic acid by McAb IIF_9D_8 follows the Michaelis-Menten kinetics. The K_m value and k_{cat} are 0.036 M and 0.598 min^{-1}, respectively, and the rate acceleration (k_{cat}/k_{uncat}) is 30500. Compared with the previous McAb 32C3 induced by polyclonal antibodies to CPA, McAb IIF_9D_8 shows higher catalytic efficiency. The catalytic antibodies with the catalytic properties similar to natural enzymes could be obtained by this approach.

The common method to produce catalytic antibodies is to use the transition-state analogue of the catalytic reaction as hapten[1,2]. According to the introduction of network theory of immune regulation[3], the binding site of the anti-idiotypic antibody can form the internal-image and mimic the structure of the antigen[3-6]. At present, this property of the anti-idiotypic antibody has been used to design anti-idiotypic catalytic antibodies which can mimic the structure of the enzymatic active site. A new approach was developed to produce catalytic antibodies by using the enzymatic active site as antigen. Some anti-idiotypic antibodies with the catalytic activities similar to natural enzymes were obtained[5-10].

Carboxypeptidase A (CPA) is a Zinc-containing exopeptidase exhibiting peptidase as well as esterase activity. Its structure and function have been well studied[11,12]. To produce anti-idiotypic catalytic antibodies with the catalytic activity similar to CPA is valuable for the study of structure-activity relationships of catalytic antibodies and the production of new catalytic antibodies. In the previous report[10], the polyclonal antibodies to CPA were used as antigen to induce the monoclonal anti-idiotypic antibody 32C3 exhibiting an esterase activity. The K_m value of the 32C3 was 0.04 M. When the concentration of McAb 32C3 reached 3.17×10^{-5} M in that

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0960-894X/99/$ - see front matter
PII: S0960-894X(99)00222-X

experimental system, the k_{cat} and the rate acceleration (k_{cat}/k_{uncat}) were 0.0123 min^{-1} and 1750, respectively. More efforts were put on how to produce anti-idiotypic catalytic antibodies which show much higher catalytic efficiency. When the monoclonal antibody which can recognize the enzymatic active site and competitively inhibit the enzymatic activity is used as antigen, it is more likely to obtain the anti-idiotypic catalytic antibody which can form the internal-image of the enzymatic active site and exhibits much higher catalytic efficiency[6]. In this report, a new monoclonal anti-idiotypic catalytic antibody IIF_9D_8 with a CPA esterase-like activity was elicited by $ID_{11}D_7$, the monoclonal competitive inhibitory antibody to CPA. Its kinetic properties were studied and the kinetic constants were determined. The K_m value was 0.036 M. When the concentration of McAb IIF_9D_8 was 2.36×10^{-6} M in the experimental system, the k_{cat} and the rate acceleration (k_{cat}/k_{uncat}) were 0.598 min^{-1} and 30500, respectively. Compared with 32C3 induced by the polyclonal antibodies to CPA, McAb IIF_9D_8 shows not only higher catalytic efficiency but also enhanced catalytic properties.

Experiments and results

The production of the monoclonal competitive inhibitory antibody to CPA : Balb/c mice were immunized with CPA from bovine pancreas (Sigma, Product No. C-0386). The hybridoma cells were produced as described[13]. The positive clones were selected by ELISA. The cells producing monoclonal antibodies were obtained by single-cell cloning with limiting dilution and injected into intraperitoneal cavity of Balb/c mice primed with liquid paraffin to obtain ascites fluid. The monoclonal antibodies were obtained after ascites fluid was purified according to the method described[14]. The effect of the various monoclonal antibodies on the enzymatic activity of CPA was determined[13]. Dixon plot represented the inhibition of catalytic activity of CPA by monoclonal antibodies to CPA (Figure1,2). The results shown in Figure 1,2 demonstrate that McAb $ID_{11}D_7$ competitively inhibits the peptidase and esterase activity of CPA with inhibitory constants 6.3×10^{-9} M and 8.8×10^{-8} M, respectively. The isotype of McAb $ID_{11}D_7$ is IgG_1, and its molecular weight is 149 KD.

The production and study of the monoclonal anti-idiotypic catalytic antibody

Production: Balb/c mice were immunized with the purified McAb $ID_{11}D_7$. The method used to produce and purify the monoclonal anti-idiotypic antibody is same as the one mentioned above. In order to screen the cells which produce anti-idiotypic antibodies showing esterase activities, HPLC was used to analyze the formation of hippuric acid and phenyllactic acid in the mixture of culture supernatant of hybridoma with hippuryl-DL-phenyllactic acid. The monoclonal anti-idiotypic antibody IIF_9D_8 with an esterase activity was obtained by analyzing 420 samples. The isotype of McAb IIF_9D_8 is IgG_1 and its molecular weight is 148 KD.

Catalytic activity: The catalytic activity of McAb IIF_9D_8 was measured by HPLC in the pH 7.4, 0.05 M Tris-HCl/0.5 M NaCl buffer with various concentrations of hippuryl-DL-phenyllactic acid. The concentration of McAb IIF_9D_8 was 2.36×10^{-6} M. Lineweaver-Burk plot showed the kinetics of hippuryl-DL-phenyllactic acid hydrolysis catalyzed by McAb IIF_9D_8 (Figure 3). The hydrolysis follows Michaelis-Menten kinetics. The kinetic constants were determined (Table 1). Ethyl *p*-hydroxy benzoate was used as a substrate to check the specificity of McAb IIF_9D_8 , but its hydrolysis catalyzed by IIF_9D_8 has not been observed yet. The result suggests that McAb IIF_9D_8 has the same substrate specificity as CPA.

Inhibition: The inhibition of the esterase activity of McAb IIF_9D_8 was studied with McAb $ID_{11}D_7$ and hydrocinnamic acid which is the competitive inhibitor of the esterase activity of CPA, respectively. Dixon plot showed that hydrocinnamic acid competitively inhibits the catalytic activity of McAb IIF_9D_8 (Figure 4), and the inhibitory constant is 8.4×10^{-6} M. After McAb IIF_9D_8 at 2.93×10^{-6} M was incubated with McAb $ID_{11}D_7$ at 2.16×10^{-6} M for 2 hours, the residual esterase activity of McAb IIF_9D_8 was measured by HPLC. 21% inhibition of the catalytic activity was observed.

Table1:The kinetic constants for the hydrolysis of hippuryl-DL-phenyllactic acid by McAb IIF_9D_8 and CPA

Catalyst	K_m(M)	k_{cat}(min^{-1})	k_{cat}/k_{uncat}
IIF_9D_8	3.6×10^{-2}	5.98×10^{-1}	3.05×10^{4}
CPA	3.46×10^{-4}	1.86×10^{3}	9.63×10^{7}

The k_{uncat} is 1.93×10^{-5} min^{-1} under this experimental conditions.

Discussion

In the approach of producing anti-idiotypic catalytic antibodies, the key step is to produce the antibody which can recognize the enzymatic active site[6]. The binding site of this antibody has structural features complementary to the active site of the enzyme, then this antibody is characterized as an inhibitor of the enzymatic reaction. When such a antibody is used as antigen, the anti-idiotypic catalytic antibody that generates the internal-image of enzymatic active site can be obtained. Antibodies are the proteins with high molecular weight, and their interaction with an enzyme should be described as a specific protein-protein interaction in which several amino acid residues of the participant proteins are involved. When containing a specific region against the active site of an enzyme, the monoclonal antibody is able to competitively inhibit the enzymatic activity[13]. Since McAb $ID_{11}D_7$ is able to competitively inhibit the activity of CPA, the binding site of McAb $ID_{11}D_7$ should recognize the enzymatic active site of CPA. When McAb $ID_{11}D_7$ was used as antigen, the monoclonal anti-idiotypic catalytic IIF_9D_8 with a CPA esterase-like activity was obtained. The result indicates that McAb $ID_{11}D_7$ has a specificity towards the enzymatic active site of CPA and is suited to be used as antigen

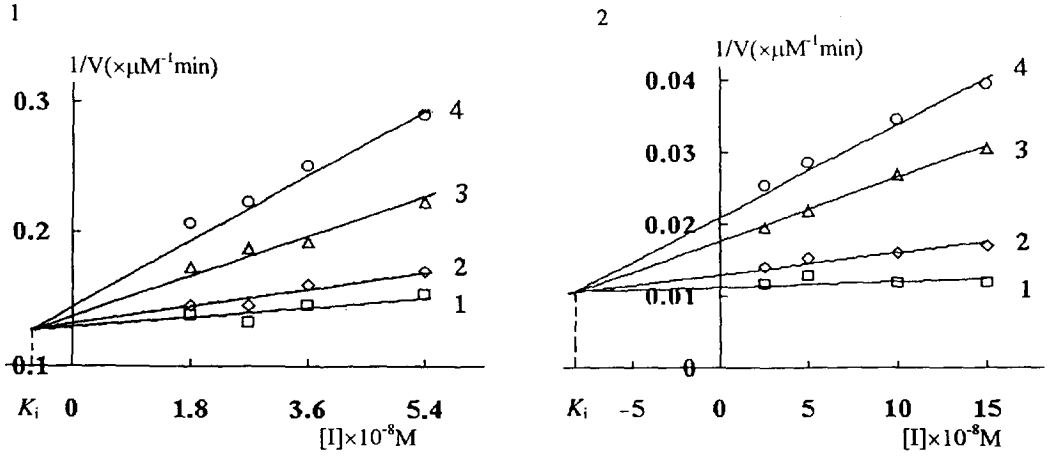

Figure 1: Dixon plot represents the inhibition of peptidase activity of CPA by McAb $ID_{11}D_7$. The concentrations of the substrate (hippuryl-L-phenylalanine) were 1, 0.8, 0.6 and 0.4 mM for lines 1-4, respectively.

Figure 2: Dixon plot represents the inhibition of esterase activity of CPA by McAb $ID_{11}D_7$, The concentrations of the substrate (hippuryl-DL-phenyllactic acid) were 1, 0.8, 0.6 and 0.4 mM for lines 1-4, respectively.

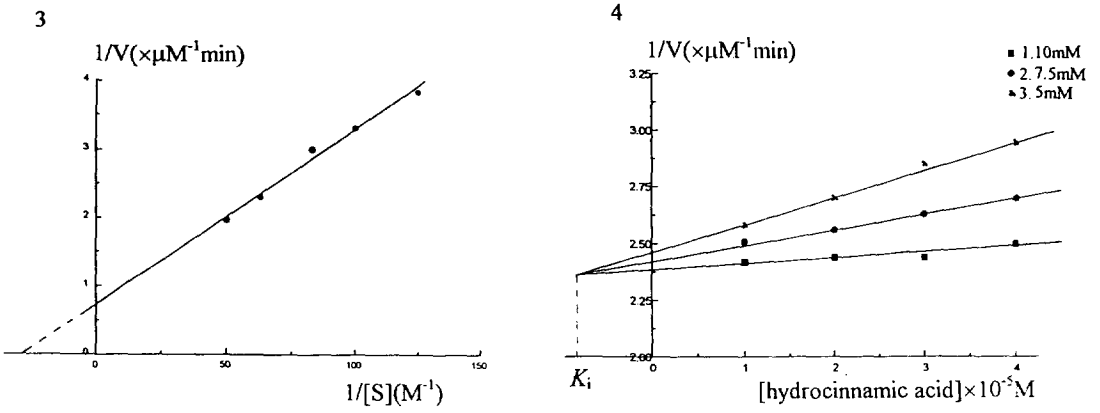

Figure 3: Lineweaver-Burk plot shows the kinetics of hippuryl-DL-phenyllactic acid hydrolysis catalyzed by mAb IIF_9D_8. The concentration of McAb IIF_9D_8 is 2.36×10^{-6}M.

Figure 4: Dixon plot shows the inhibition of esterase activity of McAb IIF_9D_8 by hydrocinnamic acid for three different substrate concentrations: 1, 10 mM; 2, 7.5 mM; 3, 5 mM.

to induce "internal-image" anti-idiotypic catalytic antibody.

The monoclonal anti-idiotypic catalytic antibody IIF_9D_8 was obtained by using $ID_{11}D_7$, the monoclonal competitive inhibitory antibody to CPA, as antigen. The hydrolysis of hippuryl-DL-phenyllactic acid catalyzed by McAb IIF_9D_8 follows the Mechaelis-Menten kinetics, and the K_m value is 0.036 M. When the concentration of McAb IIF_9D_8 is 2.36×10^{-6}M in the experimental system, the k_{cat} value is 0.598 min^{-1}. Compared with the rate of auto-hydrolysis of hippuryl-DL-phenyllactic acid, the rate acceleration (k_{cat}/k_{uncat}) is 30500. McAb IIF_9D_8 has the same specificity as CPA and its catalytic activity is competitively inhibited by hydrocinnamic acid, the competitive inhibitor of the esterase activity of CPA. The results demonstrate that McAb IIF_9D_8 forms the internal image of the active site of CPA and shows the CPA esterase-like activity. In the previous report[10], the polyclonal antibodies to CPA were used as antigen to induce the monoclonal anti-idiotypic antibody 32C3 with an esterase activity. The K_m is 0.04 M. When the concentration of McAb 32C3 reaches 3.17×10^{-5} M in the experimental system, the k_{cat} and the rate accelerationis (k_{cat}/k_{uncat}) are 0.0123 min^{-1} and 1750, respectively. A comparison of the catalytic properties of McAb IIF_9D_8 with those of 32C3 indicates that, in the same experimental system, though the concentration of the catalytic antibody IIF_9D_8 decreases an order of magnitude, the k_{cat} increases about 50 times and the rate acceleration is higher. These results suggest that McAb IIF_9D_8 exhibits higher catalytic efficiency and its catalytic properties are improved. Therefore, the anti-idiotypic catalytic antibodies with the catalytic properties similar to natural enzyme can be obtained by using the monoclonal antibody which recognizes the enzymatic active site as antigen.

In the process of screening and producing monoclonal anti-idiotypic catalytic antibodies with HPLC, several systems were used as the controls[10] to prove that the CPA esterase-like activity comes from the monoclonal anti-idiotypic catalytic antibody IIF_9D_8. Furthermore, in contrast to CPA, whose esterase activity is inhibited at the substrate concentrations above 1mM[12], the hydrolysis of hippuryl-DL-phenyllactic acid catalyzed by McAb IIF_9D_8 continues even at substrate concentrations greater than 1mM. However, the catalytic activity of McAb IIF_9D_8 can be competitively inhibited by hydrocinnamic acid, and the inhibition of the catalytic activity by McAb$ID_{11}D_7$ is 21%. Therefore, we conclude that the esterase activity is due to the monoclonal anti-idiotypic catalytic antibody IIF_9D_8 which mimic the structure of active site of CPA.

The approach of producing the anti-idiotypic antibodies to natural enzymes is valuable for the study of structure-activity relationships of catalytic antibodies and the production of new catalytic antibodies. In the structural studies of antibodies, the monoclonal antibodies provide more information than the polyclonal antibodies, therefore it is prefered to produce the monoclonal anti-idiotypic catalytic antibodies by using the monoclonal antibody against the enzymatic active site as antigen. Moreover, in the production of catalytic antibodies, a still unsolved problem is the production of catalytic antibodies showing peptidase activity. It is

possible to obtain new catalytic antibodies with peptidase activity by producing the anti-idiotypic antibodies against natural peptidase.

Conclusion

In this report, a new monoclonal anti-idiotypic catalytic antibody with a CPA-like activity was produced by using a monoclonal antibody, which can recognize the enzymatic active site, as antigen. In contrast to the previous anti-idiotypic catalytic antibody induced by polyclonal antibodies to the enzyme, this new anti-idiotypic catalytic antibody exhibits not only the esterase activity similar to CPA but also higher catalytic efficiency. Therefore, catalytic antibodies with the catalytic properties similar to natural enzyme could be obtained by this approach.

Acknowledgements This project was supported by National Science Foundation of China (29632004) and the President's Science Foundation of Peking University. We thank Mr. Guiyang Xie and Ms. Xiu Zhang for their help in experiments.

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