PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2009, 36(2): 182~189
www.pibb.ac.cn

Enzymological Characteristics of Catalytic Antibody-catalyzed Enantioselective Hydrolysis of Ibuprofen Ester in Water-in-oil Microemulsion*

YANG Gen-Sheng[1,2], QI Ying-Dan[2], OU Zhi-Min[2], YAO Shan-Jing[1]**
([1]) *Department of Chemical and Biochemical Engineering, Zhejiang University, Hangzhou* 310027, China;
[2] *College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou* 310032, China)

Abstract The asymmetric hydrolyzation of racemic ibuprofen ester is one of the most important methods for chiral separation of ibuprofen. A catalytic antibody that accelerates the rate of enantioselective hydrolysis of ibuprofen methyl ester was successfully elicited against an immunogen consisting of tetrahedral sulfate hapten attached to bovine serum albumin (BSA). The rate constant enhancement factor K_{cat}/K_{uncat} was about 1.6×10^4. The catalytic activity of the catalytic antibody in a reverse micelle reaction system based on sodium *bis* (2-ethylhexyl) sodium sulfosuccinate (AOT) in isooctane was studied. Kinetic analysis of the catalytic antibody-catalyzed reaction was found to be possible in this system. Kinetic studies showed that hydrolysis in the microemulsion system follow Michaelis-Menten kinetics. The catalytic antibody can also accelerate catalysis of S-ibuprofen methyl ester in the microemulsion system. Temperature effects, the pH profile, K_{mapp} and K_{cat} were determined. The dependence of the catalytic antibody hydrolytic activity on the w_o (molar ratio of water to surfactant) showed a bell-shaped curve, presenting a maximum at about $w_o = 21$.

Key words catalytic antibody, enantioselective hydrolysis, ibuprofen, W/O microemulsion, enzymological characteristics, *bis* (2-ethylhexyl) sodium sulfosuccinate
DOI: 10.3724/SP.J.1206.2008.00347

Catalytic antibodies, as a new class of man-made biocatalysts, have had very promising applications since they were first reported by two groups [1, 2]. To date, catalytic antibodies have shown the ability to catalyze a variety of chemical reactions, such as hydrolysis, the Diels-Alder reaction, and the retro-aldol process[3]. Catalytic antibodies have conventionally been applied to aqueous reaction systems in almost all studies, because they are thought to be soluble and stable only in the aqueous phase. However, their versatility would be expanded if reactions could also be performed in nearly anhydrous organic media, aqueous-organic biphasic media, or water-in-oil microemulsions. Reverse micelles (W/O microemulsions) are thermodynamically stable water droplets dispersed in an organic phase by means of a surfactant. One of the most important properties of reverse micelles is their ability to entrap enzymes and other biomolecules in their water droplets[4]. To date, enzymatic reactions in reverse micelles have been widely reported, but little research has been conducted on the catalytic antibody function in microemulsions [5~7]. Significant features of catalytic antibodies in organic media systems have yet to be established.

Ibuprofen, 2-(4-isobutylphenyl) propionic acid, is a widely used nonsteroidal anti-inflammatory drug that belongs to the family of 2-arylpropionic acid derivatives. The pharmaceutical activities of 2-arylpropionic acid derivatives are often dramatically dependent on the chirality of these compounds. In the case of ibuprofen, because of the asymmetric carbon in the second position, its *S*-(+)-enantiomer is known to be about 100 times more active than its *R*-(–)-enantiomer [8]. Resolution of racemic ibuprofen has been achieved by

*This work was supported by a grant from The Natural Science Foundation of Zhejiang, China (Y404353).
**Corresponding author.
Tel: 86-571-87951982, E-mail: yaosj@zju.edu.cn
Received: June 29, 2008 Accepted: August 19, 2008

enzyme-catalyzed enantioselective hydrolysis of the corresponding racemic esters, amides and nitriles [9]. However, some disadvantages, such as toxicity of ibuprofen derivatives toward microorganisms and low catalytic activity of lipases, have been observed in previous studies. Some additional new methods, such as ionic liquids[10], cellulose derivatives[11] and biphasic enzymatic membrane reactors (EMRs)[12] have emerged as applications for enzyme-catalyzed kinetic resolution.

Herein, a new biocatalyst is described, a catalytic antibody generated from the transition-state sulfate analog, which produced ibuprofen in an enantiomerically pure (*S*)-configuration from its corresponding racemic esters in a sodium bis (2-ethylhexyl) sodium sulfosuccinate (AOT)/isooctane microemulsion. The objectives of this study were to explore the enzymological characteristics of catalytic antibodies solubilized in an organic solvent in the presence of surfactant and water and some aspects of the application of catalytic antibodies as catalysts in anhydrous media.

1 Materials and methods

1.1 Materials

Racemic ibuprofen and isobutylbenzene were supplied by Quhua Group Corporation and CNPC Chemical (China) respectively. *S*- and *R*-ibuprofen were obtained from Sigma. Ibuprofen methyl ester was synthesized using the method of Lee *et al.*[13] in our laboratory. No remaining ibuprofen could be detected in the product as tested by thin-layer chromatography (TLC) and infrared spectroscopy (IR). l-ethyl-3-(3-dimethylaminopropyl) carbodiimide was purchased from Shanghai Medpep Co., Ltd. BSA, peroxidase labeled goat anti-rabbit IgG, Freund's complete and incomplete adjuvants, Sephadex G-25, and bis (2-ethylhexyl)sodium sulfosuccinate (AOT) were purchased from Sigma. Other chemicals were of G.R. grade and used without further purification. The water used was deionized and double distilled prior to use.

1.2 Preparation of the catalytic antibody

1.2.1 Hapten synthesis.

The hapten(*1*) used for immunization and antigen coating and the synthetic route for the hapten are illustrated in Figure 1. The procedure for the synthesis of the hapten was as follows.

a. Synthesis of 4-isobutylacetophenone(*3*). Acetyl chloride (22 g, 180 mmol) was added to a suspension of $AlCl_3$ (22 g, 165 mmol) in CH_2Cl_2 (200 ml) at 0℃. After the mixture was stirred at 0℃ for 10 min, isobutyl benzene (20 ml, 180 mmol) was added slowly and stirring was continued at the same temperature for 5 h. The reaction mixture was poured into a mixture of hydrochloric acid and ice water. The organic layer was separated, washed with water and brine, and dried with anhydrous Na_2SO_4. After evaporation of the solvent, the residue was distilled (170℃ at 2.7×10^3 Pa) to give 4-isobutylacetophenone (*3*, 22.5 g, 85% yield) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.91 (d, 6H, CH_3), 1.90 (m, 1H, CH), 2.53 (d, 2H, CH_2), 2.58 (s, 3H, $COCH_3$), 7.23 (d, 2H, ArH), 7.88 (d, 2H, ArH).

b. Synthesis of 1-(4-isobutylphenyl)-1-ethanol(*4*). $NaBH_4$(5.8 g, 0.16 mol) was added twice to a solution of 4-isobutylacetophenone (*3*, 14 g, 0.32 mol) in methanol (120 ml) at 0℃. The reaction was monitored by TLC. After addition of ice water (100 ml), the solution was acidified to pH3 with 30% HCl. The mixture was then stirred at room temperature for 15 min and extracted with Et_2O (3× 100 ml). The combined ether layers were washed with brine, dried over Na_2SO_4, and concentrated to give a colorless liquid (*4*, 13.3 g, 95% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.97 (d, 6H, CH_3), 1.30 (d, 3H, CH_3), 1.80 (m, 1H, CH), 2.41 (d, 2H, CH_2), 4.67 (m, 1H, CHOH), 5.13 (s, 1H, OH), 7.08 (d, 2H, ArH), 7.23 (d, 2H, ArH).

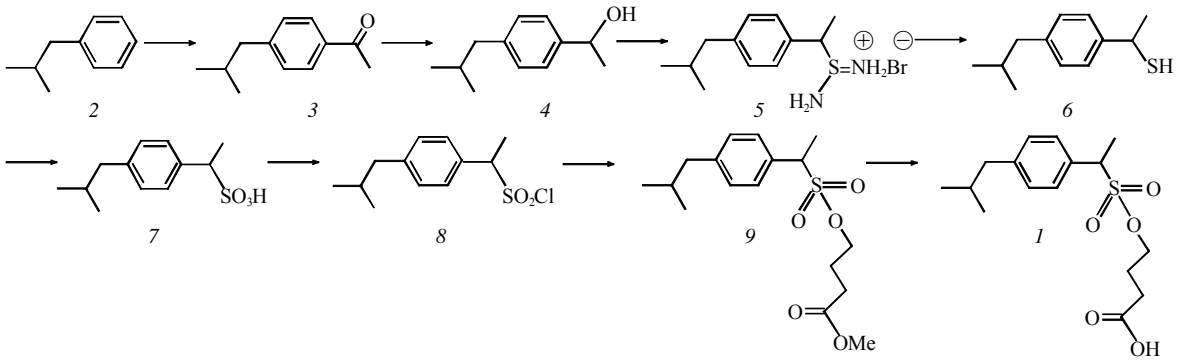

Fig. 1 Synthesis route for hapten(*1*)

c. Synthesis of the intermediate (*5*). A mixture of compound (*4*, 0.10 mol), thiourea (0.10 mol), 48% HBr (40 ml), and EtOH (50 ml) was refluxed overnight. After cooling to room temperature, the reaction mixture was concentrated in vacuo, yielding a white solid. This crude salt was used without further purification.

d. Synthesis of 1-(4-isobutylphenyl) ethanethiol (*6*). The thiourea salt (*5*, 0.10 mol) was dissolved in water (50 ml) and heated to 50℃. To this mixture, 33% NaOH solution was added dropwise until no more cloudiness developed upon addition and the pH had risen to 10. The reaction mixture was stirred overnight at 50℃. After cooling to room temperature, 30% HCl was added until a pH of 6 was reached. The reaction mixture was extracted with Et_2O (3×100 ml). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to a liquid *6* (65% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.01 (d, 6H, CH_3), 1.65 (d, 3H, $ArCHCH_3$), 2.45 (m, 2H, CH_2), 4.12 (q, 1H, ArCHCH3), 7.11 (d, 2H, ArH), 7.24 (d, 2H, ArH).

e. Synthesis of 1-(4-isobutylphenyl) ethyl sulfoacid (*7*). The thiol (*6*, 36 mmol) was dissolved in HOAc (120 ml). H_2O_2 (110 ml, 30%) was added dropwise at such a rate that the temperature remained below 32℃. After addition was complete and the thiol had reacted (according to TLC), Me_2S was added at 0℃ until no more peroxides were present. The reaction mixture was concentrated in vacuo, yielding oil. This residue was suspended in H_2O (ca. 80 ml). The water layer was washed with Et_2O (3×75 ml). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to yield the free sulfonic acid (*7*, 36% yield). ^{1}HNMR (DMSO-d_6): δ 0.90 (d, 6H, CH_3), 1.47 (d, 3H, $ArCHCH_3$), 1.62 (s, 1H, SH), 1.85 (m, 1H, CH), 2.45 (d, 2H, CH_2), 3.60 (q, 1H, ArCH), 7.05 (d, 2H, ArH), 7.28 (d, 2H, ArH).

f. Synthesis of 1-(4-isobutylphenyl) ethanesulfonyl chloride (*8*). A mixture of 1-(4-isobutylphenyl) ethyl sulfoacid (*7*, 6.8 g), $SOCl_2$ (30 ml), and DMF (2 drops) was refluxed for 2 h. Excess $SOCl_2$ was removed under a vacuum and dry benzene (20 ml) was added to remove excess $SOCl_2$ again. The crude reside was recrystallized in light petroleum to afford yellow acerate crystals (*7*, 7.0 g, yield 95%). ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.91 (d, 6H, CH_3), 1.85 (m, 1H, CH), 1.92 (d, 3H, $ArCHCH_3$), 2.45 (d, 2H, CH_2), 4.45 (d, H, CH), 7.05 (d, 2H, ArH), 7.28 (d, 2H, ArH).

g. Synthesis of methyl 4-(1-(4-isobutylphenyl) ethylsulfonyloxy) butanoate (*9*). 4-Hydroxy methyl butyrate (1.5 g, 12.5 mmol) and triethylamine (2.52 g, 25 mmol) were dissolved in CH_2Cl_2 (15 ml), and compound (*8*, 3.25 g, 12.5 mol) was partially added at 0～5℃. After complete addition, the mixture was stirred for 1 h at 0～5℃, and the reaction was continued for 1 h at room temperature. After cooling to 0℃, 1 mol/L HCl was added until pH 7 was reached, the combined organic layers were washed with saturated aqueous sodium carbonate solution and water, and dried over anhydrous magnesium sulfate. The organic layer was concentrated on a rotavapor and crude residue was passed through a silica gel column to obtain sulfonic acid ester (*9*, 1.94 g, yield 45%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.01 (d, 6H, CH_3), 1.65 (d, 3H, $ArCHCH_3$), 2.22 (m, 1H, CH), 2.25 (m, 2H, CH_2), 2.45 (d, 2H, $ArCH_2$), 2.50 (t, 2H, CH_2COOMe), 3.43 (t, 2H, OCH_2), 3.70 (s, 3H, $COOCH_3$), 4.45 (q, 1H, $ArCHCH_3$), 7.11 (d, 2H, ArH), 7.20 (d, 2H, ArH).

h. Synthesis of hapten (*1*). The sulfonic acid ester (*9*, 1.94 g, 5.6 mmol) was dissolved in ethanol (10 ml). To this mixture, NaOH aqueous solution 2 mol/L(4 ml) was added dropwise and refluxed for 30 min. The reaction mixture was concentrated in vacuo and the residue was continuously stirred for 2 h, then 1 mol/L HCl was added until pH 1～2 was reached, the mixture was filtered and dried to obtain crude product, and then recrystalized in ethanol to obtain hapten (*1*, 1.08 g, yield 56%). ^{1}H-NMR (400 MHz, CD_3OD/D_2O, 1/1): δ 1.01 (d, 6H, CH_3), 1.65 (d, 3H, $ArCHCH_3$), 2.22 (m, 1H, CH), 2.25 (m, 2H, CH_2), 2.46 (d, 2H, $ArCH_2$), 2.50 (t, 2H, CH_2COOH), 3.40 (t, 2H, OCH_2), 4.20 (q, 1H, $ArCHCH_3$), 7.16 (d, 2H, ArH), 7.25 (d, 2H, ArH). MS (ESI) *m/z* 328 (M+).

1.2.2 Synthesis of the target antigen. Hapten *1* was covalently attached to bovine serum albumin (BSA) to be used as an immunogen. The method of conjugation used was the standard EDC-promoted amide formation reaction between the carboxylic acid of hapten *1* and the δ-amine of the surface lysine of BSA. Briefly, hapten (*1*, 20 mg, 0.06 mmol) in DMF (2.2 ml) was dissolved in 4 ml of normal saline (pH 5.2). BSA (10 mg, 6.67×10^{-5} mmol), and l-ethyl-3-(3-dimethylaminopropyl) carbodiimide (30 mg) were added to the solution. The mixture was stirred at room temperature for 5 h. The mixture solution was then dialyzed against PBS buffer (pH 7.3) for six days. The formation of the BSA-hapten conjugate was confirmed

by UV/VIS spectroscopy. Quantitation of the hapten/carrier ratio afforded ratios in the range of 12∶1.

1.2.3 Immunization and purification of the catalytic antibody. The antigen was used to immunize five BALB/c mice and to generate monoclonal antibodies, following the standard protocols[14]. Five BALB/C mice were immunized with the hapten-BSA conjugate (100 μg) emulsified in complete Freund′s adjuvant. After 3 weeks, a second immunization (100 μg in incomplete Freund's adjuvant) was administered and similarly, the third immunization. Three days later, the spleen cells from the immunized mice were fused with SP2/0 according to routine procedures. Hybridomas that secreted antibodies capable of binding hapten (*1*) were identified using ELISA and binding titers in the assay were used to select one clone for further investigation. This clone was cultured in a rolling culture bottle and the monoclonal antibody was isolated by salt fractionation. A large amount of the catalytic antibody was prepared from ascites in BALB/c mice. The antibodies were sequentially purified through ammonium sulfate precipitation at various levels, protein A affinity chromatography, DEAE-52 ion exchange chromatography, and dialysis against 30 mmol/L PBS (pH 8.0). The protein concentration was measured using the Bradford method[15].

1.3 Catalytic hydrolysis of the ibuprofen methyl ester in buffer solution

In aqueous media, the hydrolysis of the ibuprofen methyl ester was carried out in a mixture that contained various substrate concentrations (*R*, *S*- and *R*-, and *S*-ibuprofen methyl esters) and purified monoclonal antibodies in 0.05 mol/L sodium phosphate buffer pH 8.0 at 37℃. The mixture solution was kept at 37℃ for the prescribed time interval and the catalytic activity was destroyed by thermal denaturation of the catalytic antibodies at 95℃ for 10 min following by rapid cooling to 0℃. The analysis of both enantiomers of ibuprofen was conducted by HPLC [16] with an Agilent Eclipse XDB-C8 column (5 μm, 4.6 mm × 250 mm; Agilent Co., USA) and a UV detector (265 nm). The mobile phase was composed of distilled water-methanol-acetonitrile (78∶17∶5 *v*/*v*/*v*, containing 80.0 mmol•L^{-1} HP-β-CD and 0.2% H_3PO_4, pH 4.5). The flow rate was set to 1.0 ml•min^{-1}.

1.4 Catalytic hydrolysis of the ibuprofen methyl ester in W/O microemulsion

In the reverse micelles, the hydrolysis reactions were performed as follows: *R*, *S*-ibuprofen methyl ester, *R*-ibuprofen methyl ester, and *S*-ibuprofen methyl ester with varying concentrations were dissolved in 500 μl of AOT/isooctane solution in different reaction vials. The reaction was started by injecting known volumes of catalytic antibody in 0.05 mol/L phosphate buffer into an isooctane solution containing 50 mmol/L AOT at 37℃. The mixture was briefly shaken until an optically clear single-phase solution was formed. Within the prescribed time interval, 20 μl samples were withdrawn in duplicate, destroyed by thermal denaturation of the catalytic antibodies at 95℃ for 10 min, and held at –20℃. The sample were diluted with 1 ml H_2O/methanol (1∶1) and analyzed by HPLC as above.

Kinetic parameters were determined using a Lineweaver-Burk plot of reaction for the antibody-catalyzed hydrolysis of substrate. The uncatalyzed rate ($K_{uncat,app}$) was determined by measuring the substrate hydrolysis rates with normal IgG of mouse instead of catalytic antibody.

As a positive control for catalytic hydrolysis of the substrates, catalytic antibody was not used in phosphate buffer. All of the data are the average of triplicate samples and are reproducible within ±15%.

2 Results and discussion

2.1 Preparation of hapten and catalytic antibodies

To elicit catalytic antibodies for the ibuprofen methyl ester hydrolysis, hapten (*1*) was designed. Hapten(*1*) was mimicked using sulfonate. This hapten was similar to the transition state analog (TSA) in structure and electrical properties. Spatially, they all have a tetrahedral structure; in electric properties, they are also extremely similar; the C—O^- and S—O^- bond lengths, as well as C=O and S=O, are all of the same magnitude. In addition, the hapten retained α-C and α-CH_3, also retained in the isobutyl structure. The carboxyl group provided the required link to the protein (BSA) used as an immunogen to raise catalytic antibodies in rats.

A preliminary assay of the hydrolytic activity of the purified antibodies was accomplished by HPLC, and 10 antibodies were found to catalyze the hydrolysis at a rate significantly above the uncatalyzed background reaction. Of these, one antibody, A5, was ultimately chosen as the active catalytic antibody. As shown in Figure 2, this catalytic antibody, which is a fully characterized lipase-like antibody produced by

hapten (*1*), can accelerate catalysis of the *S*-ibuprofen methyl ester but not the *R*-ibuprofen methyl ester.

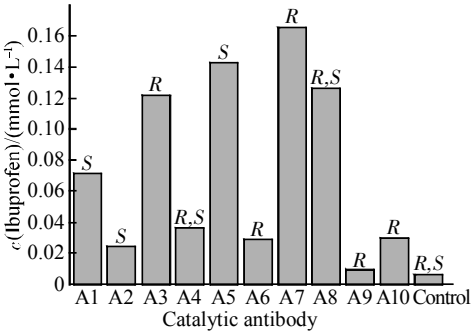

Fig. 2 Data for the initial screen of antibody-catalyzed hydrolyses of ibuprofen methyl ester

Each column represents the amount of ibuprofen methyl ester in the background reaction (control) and in the monoclonal antibody-catalyzed hydrolyses of ibuprofen methyl ester (columns A1～A10). *R,S* indicates that the product is *R,S*-ibuprofen, *R* indicates *R*-ibuprofen, *S* indicates *S*-ibuprofen. Reaction conditions: substrate, *R,S*- ibuprofen methyl ester; [substrate] /[catalytic antibody] = 25∶1 (ratio of molar concentrations); 0.2 mol/L phosphate buffer (pH 8) at 37℃; reaction time 2 h.

2.2 Catalytic antibody activity in buffer solution

Kinetic studies revealed that the catalytic antibody was found to catalyze enantioselective hydrolysis in aqueous solution, with an initial rate consistent with Michaelis-Menten kinetics. The parameters of the Michaelis-Menten kinetic equation were determined from a double-reciprocal plot of the catalytic antibody activities, shown in Figure 3b. The values of $K_{cat,app}$ and $K_{m,app}$ obtained from linear least-squares analysis were 1.01 s^{-1} and 28.31 μmol/L, respectively, at 37℃. The first order rate constant of spontaneous hydrolysis of ibuprofen methyl ester under the same conditions was 6.30×10^{-5} s^{-1}, which corresponds to a rate constant enhancement factor of 1.6×10^{4} over the uncatalyzed reaction, indicating that the catalysis reaction rates mediated by catalytic antibodies are much higher. The ratio of $K_{cat,app}/K_{uncat,app}$ reflects the acceleration of the hydrolysis reaction.

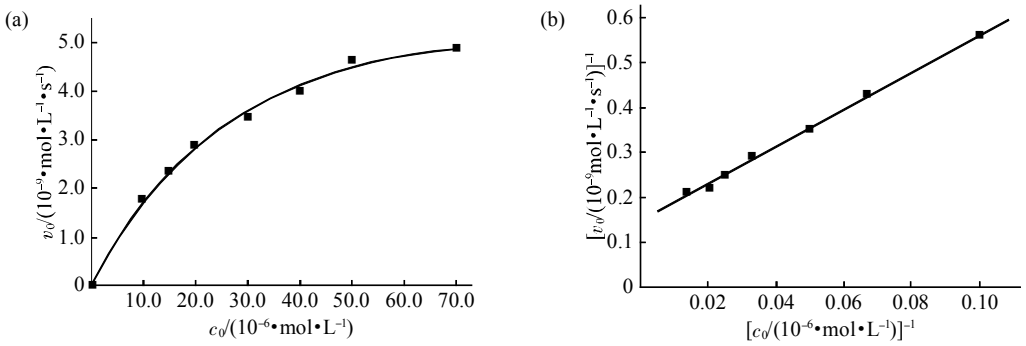

Fig. 3 Hydrolysis of ibuprofen methyl ester catalyzed by a catalytic antibody in aqueous media

(a) Initial velocity as a function of the ibuprofen methyl ester concentration. (b) Lineweaver-Burk plot of initial velocity as a function of the *S*-ibuprofen methyl ester concentration. Reaction conditions: 40 ml catalytic antibody solution (containing 0.25 μmol, 0.2 mol/L phosphate buffer, pH 8) at 37℃.

2.3 Hydrolytic activity of the catalytic antibody in W/O microemulsion

2.3.1 Effect of the w_o ratio ([H_2O] / [AOT]) on the catalytic antibody hydrolytic activity in reverse micelles.

In a microemulsion system, the effect of the water content on enzymatic catalytic behavior is the most important factor determining the reaction rates[17]. The size of the reverse micelles is controlled by the molar ratio of water to surfactant (w_o). It has been reported that varying w_o from the minimal amount necessary to stabilize the microemulsion to higher values can greatly affect the enzymatic activity.

The effect of the water content of the microemulsions is revealed in Figure 4. The reaction rate as a function of w_o follows a rather bell-shaped curve, presenting a maximum at w_o=21. This behavior is different from that observed in studies on the hydrolysis of various lipases in similar reactions[18], but is consistent with the increased molecular mass of IgG molecules. The existence of an optimal w_o for the reaction rate is probably related to conformational changes in the protein and to changes in the water structure in the hydration shell as reported by some previous investigators[19]. Many reports have revealed that most low molecular mass enzymes, such as chymotrypsin, present maximal activity at w_o values lower than 15[20], whereas enzymes with high molecular mass, such as lipoxygenase, or oligomeric enzymes such as lactate dehydrogenase, display maximal activity at a w_o between 30 and 40[20,21]. Another possible reason for the increase in w_o is that hydrolysis reactions require more water.

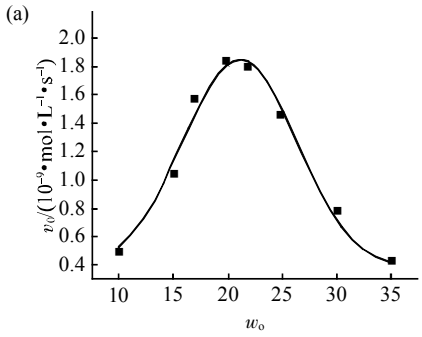

Fig. 4 Initial velocity of the catalytic antibody in reversed micelles as a function of w_o

The reaction mixture contains the catalytic antibody (6.25 μmol/L), substrate (15 μmol/L), and 100 mmol/L AOT.

2.3.2 Kinetic studies.

From these tests, it was found that the hydrolysis reactions were also following Michaelis-Menten kinetics in W/O microemulsions, as shown in Figure 5.

It was observed that the catalytic antibodies can accelerate catalysis of the *S*-ibuprofen methyl ester in the microemulsion system. Interestingly, spontaneous hydrolysis of the ibuprofen methyl ester was not observed in reverse micelles containing buffer. This result is consistent with that of Durfor *et al*.[5]

The parameters of the Michaelis-Menten kinetic equation were determined from a double-reciprocal plot (Figure 5b). For the substrate, $K_{cat,app}$ values in W/O microemulsion and in aqueous media were found to be similar, but $K_{m,app}$ values in W/O microemulsion were somewhat higher(Table 1). The increase in K_m indicates a decrease in the affinity between the substrate and the catalytic antibodies. The reason for the decrease in catalytic efficiencies ($K_{cat,app}/K_{m,app}$) and the increase in K_m could be explained by diffusion constraints on the substrates and products in the surfactant layer as well as by inhibition of the surfactant AOT.

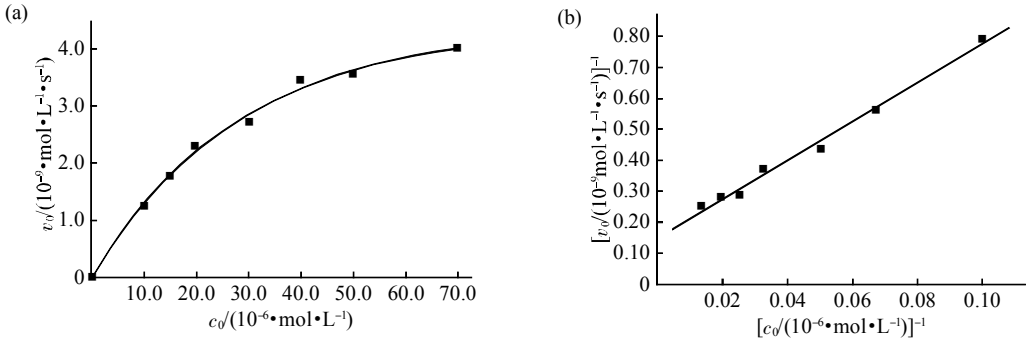

Fig. 5 Hydrolysis of ibuprofen methyl ester catalyzed by catalytic antibodies in W/O microemulsion

(a) Initial velocity as a function of the ibuprofen methyl ester concentration. (b) Lineweaver-Burk plot of the initial velocity as a function of the *S*-ibuprofen methyl ester concentration. Reaction conditions: catalytic antibody concentration 6.25 μmol/L, 50 mmol/L AOT, w_o = 21, 37℃.

Table 1 Comparison of kinetic results in aqueous solution and in W/O microemulsion

	K_{cat} /s^{-1}	K_{uncat} /s^{-1}	K_m/(μmol•L^{-1})	K_{cat} /K_{uncat}	$[\frac{K_{cat}}{K_m}]$/(mmol^{-1}•L•S^{-1})
In aqueous buffer	1.12	6.30×10^{-5}	28.31	1.6×10^{4}	39.58
In reverse micelles	1.08	–	42.28	–	25.54

–: Undetected

2.3.3 Effect of pH on catalytic antibody activity. Dependence of the catalytic antibody activity on pH in reversed micelle solutions is shown in Figure 6. The greatest activity occurs at about pH 8.0. The pH values shown are those of the aqueous buffers for which the micelle solutions were prepared.

2.3.4 Effect of temperature on catalytic antibody activity. The influence of temperature on the activity of the catalytic antibody was investigated between 10 and 50℃ (Figure 7). Micelle solutions containing substrate were preincubated at each temperature before adding

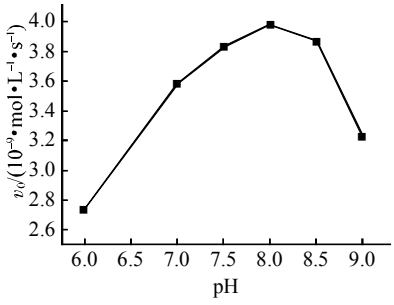

Fig. 6 Effect of pH on catalytic antibody catalytic activity in reversed micelles

Reaction conditions: catalytic antibody concentration 6.25 μmol/L, ibuprofen methyl ester concentration 70 μmol/L, AOT 50 mmol/L, w_o = 21, 37℃.

the catalytic antibody solution. The activity of the catalytic antibody reached a maximum between 30 and 40 ℃ but declined rapidly at higher temperatures. Possibly because the antibody is of animal origin, a higher temperature results in partial denaturation of the antibody protein.

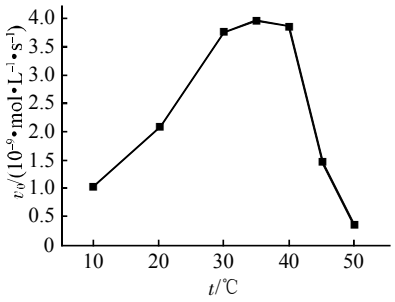

Fig. 7 Effect of temperature on catalytic antibody catalytic activity in reversed micelles

Reaction conditions: catalytic antibody concentration 6.25 μmol/L, ibuprofen methyl ester concentration 70 μmol/L, AOT 50 mmol/L, w_o=21, pH=8.0.

3 Conclusion

In this paper, a catalytic antibody that accelerates the rate of enantioselective hydrolysis of ibuprofen methyl ester was successfully elicited against hapten(*1*). This study demonstrates that enantioselective hydrolysis reactions can be catalyzed by antibodies generated against the carefully designed haptens. The experiments revealed that a catalytic antibody retains its catalytic function after its entrapment in an AOT/isooctane reverse micelle system. The enantioselectivity was total for the *S*-enantiomer of ibuprofen in this study. Optimal antibody activity was observed at a w_o value of 21, consistent with the increased molecular mass of IgG molecules. Although the catalytic activity of this antibody is lower than natural enzymes, the ability of the antibodies to preserve their catalytic activity in reverse micelles should significantly expand the versatility of antibodies as catalysts in biotechnological processes.

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油包水微乳液中抗体酶催化布洛芬酯选择性水解的酶学特性 *

杨根生[1,2)]　戚映丹[2)]　欧志敏[2)]　姚善泾[1)**]
([1)]浙江大学生物与化学工程与生物工程学系，杭州 310027；[2)]浙江工业大学药学院，杭州 310032)

摘要　根据过渡态理论设计和合成了能诱导产生催化选择性水解布洛芬甲酯的催化抗体的四面体硫酸盐半抗原，并与牛血清白蛋白(BSA)偶联制备成免疫源，通过免疫手段成功筛选出具有加速选择性水解生成 *S*- 布洛芬的特异性催化抗体．其 $K_{cat,app}/K_{uncat,app}$ 达 1.6×10^4．进一步地将催化抗体运用到 W/O 微乳体系(反胶束)中进行布洛芬酯的选择性水解研究，其动力学研究证明其催化过程同样遵循 Michaelis-Menten 方程．考察了 pH 值和温度对催化初速度影响，w_o(体系中水和琥珀酸二辛酯磺酸钠(AOT)的摩尔比) 对催化初速度影响呈现为钟罩型，最适的 w_o 为 21．

关键词　催化抗体，选择性水解，布洛芬，W/O 微乳，酶学，琥珀酸二辛酯磺酸钠
学科分类号　Q814，O64，Q55　　**DOI:** 10.3724/SP.J.1206.2008.00347

* 浙江省自然科学基金资助项目(Y404353).
** 通讯联系人.
Tel: 0571-87951982, E-mail: yaosj@zju.edu.cn
收稿日期：2008-06-29，接受日期：2008-08-19