ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 18 (2002) 81–87

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Characteristics of magnetic nanoparticles-bound YADH in water/AOT/isooctane microemulsions

Min-Hung Liao, Dong-Hwang Chen*

Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan, ROC

Received 27 March 2002; received in revised form 18 April 2002; accepted 7 May 2002

Abstract

The yeast alcohol dehydrogenase (YADH) covalently bound onto Fe_3O_4 magnetic nanoparticles was performed in the NADH-containing water-in-oil microemulsions of water/AOT/isooctane. Both water and NADH were present in the aqueous phase of microemulsion solution and on particle surface. The ratio of the equilibrium water content on particle surface to that in the aqueous phase of microemulsion solution (i.e. aquaphilicty) was about 0.39 and independent of the molar ratio of water to AOT (ω_0). The thickness of aqueous film on particle surface was estimated to be 3.1–5.7 nm, increasing with increasing the ω_0 value from 10 to 25. At a constant NADH amount, the concentration of NADH in the aqueous phase of microemulsion solution was not significantly affected by the ω_0 value. In addition, the bound YADH showed excellent storage stability and good thermal stability in the microemulsion system. At 25 °C for 700 h and 75 °C for 30 min, its residual activities were 78 and 56%, respectively. The specific activity of bound YADH in the microemulsion system was 40% of that in aqueous solution. The maximum specific activity of bound YADH (V_{max}), and the Michaelis constants for NADH (K_m^A) and 2-butanone (K_m^B) were determined to be 0.0306 μmol/min mg, 0.0589 mM, and 0.1476 M, respectively.

Keywords: YADH; Magnetic nanoparticles; Microemulsions; Stability; Distribution

1. Introduction

During the past decade, many efforts were made on nanoparticles because of their unusual physical and chemical properties owing to extremely small size and large specific surface area [1–5]. Magnetic nanoparticles have received considerable attention because of their potential applications in the immobilization of proteins and enzymes [6–11], bioseparation [12–15], immunoassays [15–17], drug delivery [15,18–20], biosensors [21], protein assay [22] and so on. Using magnetic nanoparticles as the support of immobilized enzymes has the following advantages: (i) higher specific surface area was obtained for the binding of a larger amount of enzymes, (ii) lower mass transfer resistance and less fouling, and (iii) the immobilized enzymes can be selectively separated from a reaction mixture by the application of a magnetic field [6].

Alcohol dehydrogenase, which catalyzes the oxidation of alcohols and the reduction of carbonyl compounds such as aldehydes and ketones, has attracted attention because of its potential applications in the production of various starting materials and intermediates in chemical industry, the synthesis of chiral compounds, the regeneration of coenzymes NAD(P) and NAD(P)H, and biosensors [23–25]. Unfortunately, its stability is poor, particularly in the organic media, and hence, limits its practical applications [26–29].

* Corresponding author. Tel.: +886-6-2757575x62680; fax: +886-6-2344496.
E-mail address: chendh@mail.ncku.edu.tw (D.-H. Chen).

1381-1177/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S1381-1177(02)00062-0

Recently, we have successfully immobilized the yeast alcohol dehydrogenase (YADH) onto Fe_3O_4 magnetic nanoparticles and found that it showed a 10-fold increased stability in an aqueous solution [30,31]. In this work, we attempted to further investigate the performance of the YADH-bound magnetic nanoparticles in organic media. To avoid serious particle agglomeration, control the water content and add the coenzyme NADH, the water-in-oil microemulsions of water/AOT/isooctane was used as the organic medium. Such a nanoparticle-containing microemulsion system for the enzyme reaction was seldom mentioned. The stability and kinetic behaviors of bound YADH were investigated. The distributions of water and NADH in microemulsion solution and on particle surface were also examined.

2. Materials and methods

2.1. Materials

Crystallized and lyophilized alcohol dehydrogenase (EC1.1.1.1) from baker's yeast (no. A-3263), β-nicotinamide adenine dinucleotide, reduced form (NADH, N-8129), sodium di-2-ethylhexylsulfosuccinate (AOT) and carbodiimide were purchased from Sigma (St. Louis, MO). Bio-Rad reagent for protein assay was obtained from Bio-Rad Lab. (Hercules). Ferric chlorides, 6-hydrate and ferrous chloride tetrahydrate were the products of J.T. Baker (Phillipsburg) and Fluka (Buchs), respectively. 2-Butanone was an analytic grade reagent of Ferak (Germany). Ammonium hydroxide (29.6%) was supplied by TEDIA (Fairfield). Tris(hydroxymethyl)aminomethane (Tris) and hydrochloric acid were the guaranteed reagents of E. Merck (Darmstadt). The water used throughout this work was the reagent-grade water produced by Milli-Q SP ultra-pure-water purification system of Nihon Millipore Ltd., Tokyo. All other chemicals were the guaranteed or analytic grade reagents commercially available and used without further purification.

2.2. Preparation of YADH-bound magnetic nanoparticles

The YADH-bound magnetic nanoparticles were prepared according to our recent work [30,31]. First, magnetic nanoparticles Fe_3O_4 were prepared by co-precipitating Fe^{2+} and Fe^{3+} ions by ammonia solution and treating under hydrothermal conditions. The ferric and ferrous chlorides (molar ratio 2:1) were dissolved in water at 0.3 M. Chemical precipitation was achieved at 25 °C under vigorous stirring by adding NH_4OH (15.6 M). During the reaction process, the pH was maintained at about 10. The precipitates were heated at 80 °C for 30 min, then washed several times with water and ethanol, and finally, dried in a vacuum oven at 70 °C.

For the binding of YADH, 100 mg magnetic nanoparticles were first added to 2 ml buffer A (0.003 M phosphate, pH 6, 0.1 M NaCl). Then, the reaction mixture was sonicated for 10 min after adding 0.5 ml of carbodiimide solution (0.025 g/ml in buffer A). Finally, 2.5 ml YADH (2 mg/ml in buffer A) was added and the reaction mixture was sonicated for 30 min. The temperature of binding process was fixed at 4 °C. The YADH-bound magnetic nanoparticles were recovered from the reaction mixture by placing the bottle on a permanent magnet with a surface magnetization of 3000 gauss. The magnetic particles settled within 1–2 min. The binding efficiency was estimated to be almost 100% via the measurement of the unbound protein content in the supernatant after binding process by the colorimetric method at 595 nm using the Bio-Rad reagent for protein assay with bovine serum albumin as the standard. The precipitates were washed with buffer A then dried in a vacuum oven at 25 °C, and then directly used for the measurements of activity and stability.

The resultant YADH-bound magnetic nanoparticles had a mean diameter of 11.3 nm, a YADH/Fe_3O_4 ratio of 0.05/1 (w/w), and a saturation magnetization of 61 emu/g at 25 °C. In 0.1 M Tris–HCl buffer (pH 8.0, 0.1 M NaCl), the bound YADH retained 62% activity and showed a 10-fold increased stability [30,31].

2.3. Measurement of the distributions of water and NADH

When the YADH-bound magnetic nanoparticles were mixed by vortex with clear microemulsion solution, a black suspension solution was formed. All nanoparticles should be completely entrapped into water cores of AOT reverse micelles due to the surfactant effect and the hydrophilic surface of

YADH-bound magnetic nanoparticles. Although the nanoparticle-impregnated micelles were much heavier than those empty micelles, the time required for the complete sedimentation of YADH-bound magnetic nanoparticles was several days. So, the suspension essentially might be regarded as a stable system during the following measurements.

The distributions of water and NADH in microemulsion solution and on particle surface (Fe_3O_4 or YADH-bound Fe_3O_4) at various ω_0 values were determined by measuring their concentration changes in the microemulsion solution after being mixed with magnetic nanoparticles for a specified time. The samples were withdrawn after liquid–solid separation via a permanent magnet. A buffer B (0.1 M Tris–HCl, pH 8.0) was used as the aqueous phase of microemulsions and the temperature was fixed at 25 °C. The water content and NADH concentration in microemulsion solution were measured using a Karl-Fischer moisture meter (MKC-500; Kyoto Electronics) and following the decreased absorbance at 340 nm on a Hitachi U-3000 spectrophotometer, respectively. The water content and NADH concentration on particle surface were estimated from the mass balance law.

2.4. Activity and stability measurements

The activity of bound YADH in the water-in-oil microemulsion of water/AOT/isooctane was determined by measuring the initial reduction rate of 2-butanone by YADH at 25 °C following the decrease of NADH concentration at 340 nm. The buffer B was used as the aqueous phase of microemulsions and ω_0 value was fixed at 25. Generally, 5 ml microemulsion solution was first added to the test tube containing 100 mg YADH-bound magnetic nanoparticles. After mixing by vortex, 5 ml NADH-containing microemulsion solution was added and mixed. After 5 min, the distributions of water and NADH reached equilibrium and 54 μl 2-butanone was added to the microemulsion system and mixed to start the reaction. After several minutes, the microemulsion solution was separated from the magnetic nanoparticles via a permanent magnet then used for the analysis of NADH concentration.

In this study, the activity of bound YADH was measured at 0.1 M AOT, 0.06 M 2-butanone, 0.5 mg YADH/ml, and 0.2 mM NADH based on the overall volume of microemulsion solution. Assuming the distribution of water and NADH remained at equilibrium during the reaction, the activity of bound YADH was estimated according to the following equation:

$$\text{activity} = \frac{[\text{NADH}]_0(A_e - A_t)V}{A_e t} \quad (1)$$

where $[\text{NADH}]_0$ is the NADH concentration in microemulsion solution before contacting with YADH-bound magnetic nanoparticles. V and t denote the volume of microemulsion solution and reaction time, respectively. A_e and A_t represent the absorbances of microemulsion solution at 340 nm at time 0 and t, respectively, since the reaction was started.

The storage stability of the bound YADH in the water-in-oil microemulsion of water/AOT/isooctane was examined by assaying their residual activities at 25 °C after being incubated at 25 °C for a required period. The thermal stability was investigated by measuring its residual activities at 25 °C after being incubated at the desired temperatures for 30 min.

2.5. Determination of kinetic parameters

The kinetic parameters of bound YADH were determined by measuring its activities in the water-in-oil microemulsion at 25 °C. The concentrations of NADH, 2-butanone, YADH in the reaction mixture were 0.10–0.25 mM, 0.06–0.15 M, and 0.5 mg/ml, respectively.

3. Results and discussion

3.1. Distributions of water and NADH

Since the preliminary experiment indicated that the absorbance at 340 nm of microemulsion solution decreased after pure Fe_3O_4 or YADH-bound Fe_3O_4 nanoparticles were added, the distributions of water and NADH were investigated first. It was found that the distribution of water reached equilibrium within 5 min at $\omega_0 = 10$–25. Table 1 shows the equilibrium water content on the surface of YADH-bound Fe_3O_4 nanoparticles and that in microemulsion solution and their ratios (i.e. aquaphilicity [32]) at various ω_0 values. It indicated that both the water contents in microemulsion solution and on particle surface increased with increasing ω_0 value, while the aquaphilicity was

Table 1
Effect of ω_0 value on the distribution of water[a]

ω_0	Water content on particle surface (μl)	Water content in microemulsion solution (μl)	Aquaphilicity	Thickness of aqueous film on particle surface (nm)
10	49	131	0.38	3.1
15	79	191	0.42	4.2
20	89	271	0.33	4.5
25	133	317	0.42	5.7

[a] Microemulsion solution: 10 ml, YADH-bound Fe_3O_4 nanoparticles: 100 mg, [AOT] = 0.1 M.

independent of ω_0 value and had an average value of 0.39. Almost same result was observed for the case using pure Fe_3O_4 nanoparticles, revealing the binding of YADH did not significantly change the aquaphilicity of Fe_3O_4 nanoparticles. In addition, according to the water content on particle surface and the amount of YADH-bound Fe_3O_4 nanoparticles, the thickness of aqueous film on particle surface could be estimated to be 3.1–5.7 nm at ω_0 = 10–25 as listed in Table 1.

The distribution of NADH in the microemulsion system might be referred to the distribution of water and its association with YADH and adsorption on Fe_3O_4 nanoparticles. At [NADH] = 0.2 mM, the time courses for the retention percentage of NADH in microemulsion solution at various ω_0 values were shown in Fig. 1. At ω_0 = 25, the distribution of NADH reached equilibrium within 5 min. With decreasing the ω_0 value, the time required for the distribution of NADH to reach equilibrium increased. Since the distribution of water reached equilibrium within 5 min at ω_0 = 10–25, the longer equilibrium time required for the distribution of NADH at $\omega_0 < 25$ was not resulted from the distribution of water and could be referred to the association of NADH with YADH and the adsorption of NADH on the surface of Fe_3O_4 nanoparticles. It implied that the transfer of NADH from its own micelles to those already occupied by YADH-bound nanoparticles was difficult relatively. This might be resulted by the low water content which might not provide sufficient bound water. In addition, almost same result was observed for the case using pure Fe_3O_4 nanoparticles, revealing the amount of NADH associated with YADH was much less than those adsorbed on Fe_3O_4 nanoparticles and dissolved in the aqueous film on particle surface.

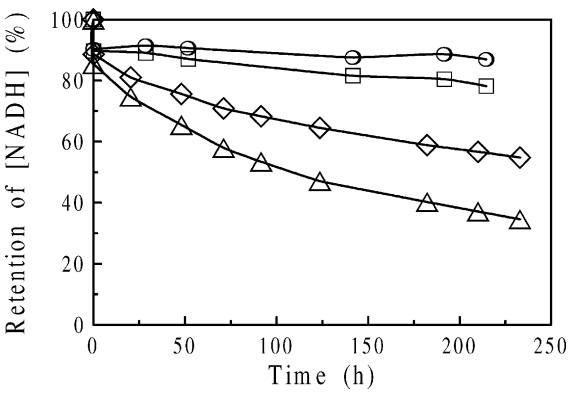

Fig. 1. The time courses for the retention percentage of NADH in microemulsion solution at ω_0 = 10 (△), 15 (◇), 20 (□), 25 (○). The initial concentration of NADH was 0.2 mM based on the overall volume of microemulsion solution.

Table 2 indicates the concentration of NADH in the aqueous phase of microemulsion solution and that on particle surface from the mass balance law (based on the volume of aqueous film) at various ω_0 values at 210 h. It was found that the concentrations of NADH in the aqueous phases of microemulsion solutions were not significantly affected by ω_0 value and they were clearly smaller than those on particle surface. However, with increasing ω_0 value, the concentrations of

Table 2
Effect of ω_0 value on the distribution of NADH[a]

ω_0	[NADH] in the aqueous phase of microemulsion solution (mM)	[NADH] on particle surface based on the volume of aqueous film (mM)
10	7.1	62
15	8.9	29
20	8.9	18
25	8.0	11

[a] Microemulsion solution: 10 ml, YADH-bound Fe_3O_4 nanoparticles: 100 mg, [AOT] = 0.1 M, [NADH] = 0.2 mM based on the overall volume of microemulsion solution.

NADH on particle surface decreased and approached those in the aqueous phase of microemulsion solutions. In fact, the concentration of NADH on particle surface included the amount of NADH dissolved in the aqueous film on particle surface, adsorbed on particle surface, and associated with YADH. Hence, the higher NADH concentration on particle surface than in the aqueous phase of microemulsion solution could be attributed to the contribution from the adsorption of NADH on particle surface and the association of NADH with YADH. With the increase in ω_0 value, the volume of aqueous solution increased and the sum of the percentage of NADH dissolved in the aqueous phase of microemulsion and that in the aqueous film on particle surface increased. In addition, assuming the concentration of NADH in the aqueous phase of microemulsion solution was the same as that in the aqueous film on particle surface, it could be deduced that the equilibrium concentration of NADH in the liquid phase of microemulsion system remained constant when there were adsorbed NADH on particle surface. Thus, it was suggested that the thermodynamically activity of adsorbed NADH could be considered as unity and the concentration of NADH in the liquid phase related to the solubility or the equilibrium constant.

3.2. Activity and stability

It is known that an enzyme usually exhibits its maximum activity at a water content just equal to that required for the bound water. As stated above, in this work, the long equilibrium time of NADH at ω_0 = 10–20 implied the water contents might be less than that required for the bound water of the nanoparticle-containing microemulsion system under these conditions. So, the activity and stability of bound YADH in the water-in-oil microemulsions of water/AOT/isooctane were examined at $\omega_0 = 25$. The specific activity of bound YADH in the microemulsion system at 0.1 M AOT, 0.06 M 2-butanone, 0.5 mg YADH/ml, 0.2 mM NADH, and 25 °C was measured to be 8 nmol/min mg. Under the same temperature and substrate and NADH concentrations, the specific activity of bound YADH in aqueous buffer B was 20 nmol/min mg. The lower specific activity of bound YADH in the microemulsion system might be attributed to the low water content in the reaction medium. In an organic medium with low water content, the enzyme had low flexibility and, therefore, its catalytic activity was diminished due to the impairment in the interplay of protein groups with the surrounding medium [33,34]. In spite of the decreased activity, however, the residual activity of the bound YADH in the microemulsion system should be reasonable compared to that in aqueous solution.

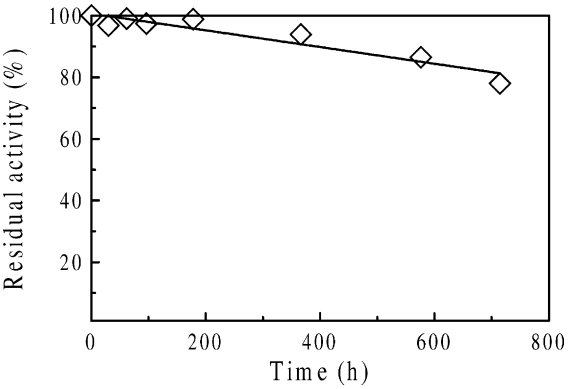

Fig. 2. Storage stability of bound YADH at 25 °C. The activity measurement was performed in 10 ml microemulsion solution at 0.1 M AOT, 0.2 mM NADH, 0.1 M 2-butanone, 25 °C, and $\omega_0 = 25$. The concentrations of bound YADH was 0.5 mg/ml. The initial 100% absolute values of activity for bound YADH was 8 nmol/min mg.

Fig. 2 shows that the storage stability of bound YADH in the water-in-oil microemulsions of water/AOT/isooctane at $\omega_0 = 25$ and 25 °C. After 700 h, the residual activity of bound YADH was 78%. According to the literatures [28,29], the free YADH in the water-in-oil microemulsions of water/AOT/isooctane lost most of its activity within 1 h, indicating very poor stability. So, the excellent stability of bound YADH might be attributed due to the fixation of YADH molecules on the surface of magnetic nanoparticles, preventing the autodigestion and thermal inactivation of enzyme and decreasing the denaturation or deactivation of enzyme due to the hydrophobic and electrostatic interactions with AOT.

The thermal stability of bound YADH in the water-in-oil microemulsion system at an incubation temperature of 25–75 °C for 30 min were indicated in Fig. 3. At an incubation temperature of 75 °C, the bound YADH still had a residual activity of 56%. This revealed clearly that the bound YADH in the water-in-oil microemulsions of water/AOT/isooctane had good thermal stability.

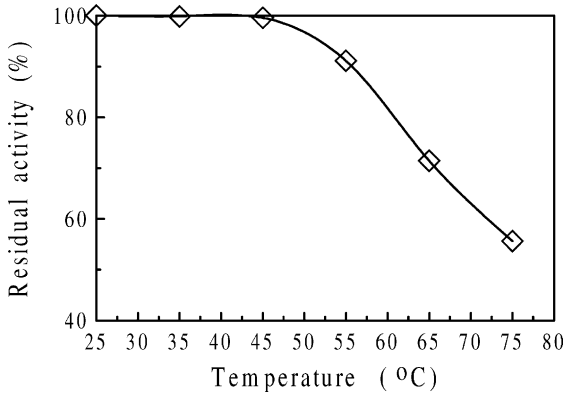

Fig. 3. Thermal stability of bound YADH. The activity measurement was performed in 10 ml microemulsion solution at 0.1 M AOT, 0.2 mM NADH, 0.1 M 2-butanone, and $\omega_0 = 25$. The concentrations of bound YADH was 0.5 mg/ml. The initial 100% absolute values of activity for bound YADH was 8 nmol/min mg at 25 °C.

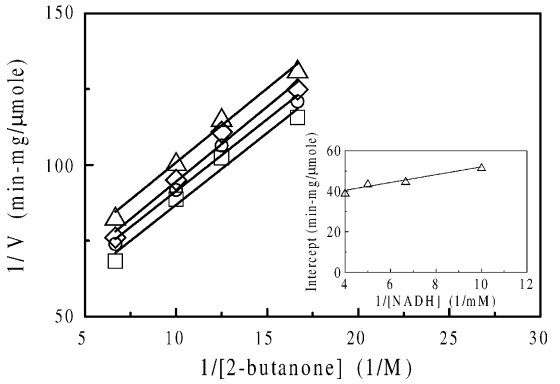

Fig. 4. Plots of $1/V$ against 1/[2-butanone] for the reduction of 2-butanone by bound YADH. The inset is the plot of the intercepts of the parallel lines against 1/[NADH]. The activity measurement was performed in 10 ml microemulsion solution at 0.1 M AOT, 25 °C, and $\omega_0 = 25$. [NADH] = 0.1 (△), 0.15 (◇), 0.2 (○), and 0.25 (□) mM. The concentration of bound YADH was 0.5 mg/ml.

According to the above, it could be concluded that the bound YADH could be performed in the microemulsion system with excellent stability and reasonable specific activity.

3.3. Kinetic parameters

Alcohol dehydrogenase has been known to operate usually by a random-order equilibrium mechanism or a compulsory-order mechanism, with one ternary complex [35]. The common rate equation can be expressed as:

$$V = \frac{V_{\max}}{1 + (K_{\mathrm{m}}^{\mathrm{A}}/a) + (K_{\mathrm{m}}^{\mathrm{B}}/b) + (K_{\mathrm{S}}^{\mathrm{A}} K_{\mathrm{m}}^{\mathrm{B}}/ab)} \quad (2)$$

where a and b are the concentration of NADH and 2-butanone, respectively; V and $V_{\max}$ the specific activity and maximum specific activity, respectively; $K_{\mathrm{m}}^{\mathrm{A}}$ and $K_{\mathrm{m}}^{\mathrm{B}}$ are the Michaelis constants for NADH and 2-butanone, respectively; $K_{\mathrm{S}}^{\mathrm{A}}$ is dissociation constant of YADH–NADH. Taking the reciprocals of Eq. (2) and rearranging,

$$\frac{1}{V} = \left(\frac{K_{\mathrm{m}}^{\mathrm{B}} + (K_{\mathrm{S}}^{\mathrm{A}} K_{\mathrm{m}}^{\mathrm{B}}/a)}{V_{\max}}\right)\frac{1}{b} + \left(\frac{1 + (K_{\mathrm{m}}^{\mathrm{A}}/a)}{V_{\max}}\right) \quad (3)$$

While conducting the experiments in the microemulsion system, a plot of $1/V$ against 1/[2-butanone] at a series of NADH concentrations was found to be a series of parallel lines, as shown in Fig. 4. The intercepts of parallel lines in Fig. 4 were further plotted against 1/[NADH] and yielding a straight line, as shown in the inset in Fig. 4. This revealed that the apparent $K_{\mathrm{S}}^{\mathrm{A}} = 0$ and the reduction rate of 2-butanone by bound YADH in the microemulsion system with NADH as coenzyme can be satisfactorily described by the following equation:

$$V = \frac{V_{\max}}{1 + (K_{\mathrm{m}}^{\mathrm{A}}/a) + (K_{\mathrm{m}}^{\mathrm{B}}/b)} \quad (4)$$

The three kinetic parameters in Eq. (4) were found to be: $V_{\max} = 0.0306$ μmol/min mg, $K_{\mathrm{m}}^{\mathrm{A}} = 0.0589$ mM, and $K_{\mathrm{m}}^{\mathrm{B}} = 0.1476$ M.

If the association of enzyme and coenzyme contains an irreversible step before substrate is bound, e.g.

$$\mathrm{E} + \mathrm{A} \leftrightarrow \mathrm{EA} \rightarrow (\mathrm{EA}^{*}) \overset{\mathrm{B}}{\leftrightarrow} \mathrm{EAB} \quad (5)$$

the apparent $K_{\mathrm{S}}^{\mathrm{A}}$ will be zero, and the term $K_{\mathrm{S}}^{\mathrm{A}} K_{\mathrm{m}}^{\mathrm{B}}/ab$ in Eq. (2) will vanish [35]. Therefore, the association of YADH and NADH could have proceeded with an irreversible reaction before the substrate was bound when performed in the microemulsion system. Since the association of YADH and NADH is relatively faster than the binding of substrate and the complex of YADH and NADH (EA) may become another new complex (EA^{*}). Thus, the simplification of the

rate equation from Eqs. (2)–(4) for the reduction of 2-butanone by bound YADH in microemulsion system might be explained.

4. Conclusions

YADH was covalently bound onto Fe_3O_4 magnetic nanoparticles and its performance in the water-in-oil microemulsion of water/AOT/isooctane was investigated. Such a nanoparticle-containing microemulsion system for the enzyme reaction was seldom examined before. It was found that water and NADH were present in the aqueous phase of microemulsion solution and on particle surface. The corresponding phenomena were investigated, including the effects of ω_0 value on the aquaphilicty of Fe_3O_4 nanoparticles, the thickness of aqueous film on particle surface, and the NADH concentration in the aqueous phase of microemulsion solution. In addition, the bound YADH exhibited excellent storage stability and good thermal stability. The residual activity of bound YADH in the microemulsion system was lower than that in aqueous solution, but it was reasonable. The kinetic parameters, including maximum specific activities and Michaelis constants, were also determined. This work revealed the bound YADH could be performed in the microemulsion system, and should be helpful for the practical application of YADH in organic media.

Acknowledgements

This work was performed under the auspices of the National Science Council of the Republic of China, to which the authors wish to express their thanks.

References

[1] J.H. Fendler, Nanoparticles and Nanostructured Films: Preparation, Characterization and Applications, Wiley, New York, 1998.
[2] G. Schimid, Clusters and Colloids: From Theory to Application, Wiley, New York, 1994.
[3] P.V. Kamat, Chem. Rev. 93 (1993) 267–300.
[4] B.C. Gates, Chem. Rev. 95 (1995) 511–522.
[5] M.L. Wu, D.H. Chen, T.C. Huang, J. Coll. Interf. Sci. 243 (2001) 102–108.
[6] P.J. Halling, P. Dunnill, Enzyme Microb. Technol. 2 (1980) 2–10.
[7] M.Y. Arica, H. Yavuz, S. Patir, A. Denizli, J. Mol. Catal. B. Enzymatic 11 (2000) 127–138.
[8] H. Kobayashi, T. Matsunaga, J. Coll. Interf. Sci. 141 (1991) 505–511.
[9] A. Kondo, H. Fukuda, J. Ferment. Bioeng. 84 (1997) 337–341.
[10] R.V. Mehta, R.V. Upadhyay, S.W. Charles, C.N. Ramchand, Biotechnol. Tech. 11 (1997) 493–496.
[11] M. Koneracká, P. Kopèanský, M. Antalík, M. Timko, C.N. Ramchand, D. Lobo, R.V. Mehta, R.V. Upadhyay, J. Magn. Magn. Mater. 201 (1999) 427–430.
[12] M. Reetz, A. Zonta, V. Vijayakrishnan, K. Schimossek, J. Mol. Catal. A. Chem. 134 (1998) 251–258.
[13] S.V. Sonti, A. Bose, J. Coll. Interf. Sci. 170 (1995) 575–585.
[14] X.D. Tong, B. Xue, Y. Sun, Biotechnol. Prog. 17 (2001) 134–139.
[15] T.M. Cocker, C.J. Fee, R.A. Evans, Biotechnol. Bioeng. 53 (1997) 79–87.
[16] W. Schütt, C. Grüttner, U. Häfeli, M. Zborowski, J. Teller, H. Putzar, C. Schümichen, Hybridoma 16 (1997) 109–117.
[17] K. Löster, S. Seidel, D. Kirstein, F. Schneider, F. Noll, J. Immunol. Meth. 148 (1992) 41–47.
[18] F. Sauzedde, A. Elaissari, C. Pichot, Macromol. Symp. 151 (2000) 617–623.
[19] W. Schütt, C. Grüttner, J. Teller, F. Westphal, U. Häfeli, B. Paulke, P. Goetz, W. Finck, Artif. Organs 23 (1999) 98–103.
[20] S.R. Rudge, T.L. Kurtz, C.R. Vessely, L.G. Catterall, D.L. Williamson, Biomaterials 21 (2000) 1411–1420.
[21] A.R. Varlan, J. Suls, P. Jacobs, W. Sansen, Biosens. Bioelectron. 10 (1995) 15–19.
[22] T.N. Krogh, T. Berg, P. Højrup, Anal. Biochem. 274 (1999) 153–162.
[23] J.B. Jones, Tetrahedron 42 (1986) 3351–3403.
[24] W. Hummel, M.R. Kula, Eur. J. Biochem. 184 (1989) 1–13.
[25] A.K. Williams, J.T. Hupp, J. Am. Chem. Soc. 120 (1998) 4366–4371.
[26] H. Ooshima, Y. Genko, Y. Harano, Biotechnol. Bioeng. 23 (1981) 2851–2862.
[27] B. Orlich, H. Berger, M. Lade, R. Schomäcker, Biotechnol. Bioeng. 70 (2000) 638–646.
[28] S. Sarcar, T.K. Jain, A. Maitra, Biotechnol. Bioeng. 39 (1992) 474–478.
[29] D.H. Chen, H.H. Chen, T.C. Huang, J. Chem. Eng. Jpn. 28 (1995) 551–555.
[30] M.H. Liao, D.H. Chen, Biotechnol. Lett. 23 (2001) 1723–1727.
[31] D.H. Chen, M.H. Liao, J. Mol. Catal. B. Enzymatic 16 (2002) 283–291.
[32] M. Reslow, P. Adlercreutz, B. Mattiasson, Eur. J. Biochem. 172 (1988) 573–578.
[33] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 3194–3201.
[34] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 8017–8021.
[35] M. Dixon, E.C. Webb, C.T.R. Thorne, K.F. Tipton, Enzymes, Longman, London, 1979.