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Lipase-catalyzed esterification processing in natural polymer containing microemulsion-based organogel systems

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1. Introduction

Microemulsions gelled by the aid of natural polymers, i.e. microemulsion-based organogels (MBGs), have become of interest as novel tools for enzyme immobilization in hydrophobic solvents [1,2]. Sodium bis (2-ethylhexyl) sulfosuccinate (AOT) is frequently employed as an amphiphile for stable MBG formation.

The enzyme activity in MBG has been influenced by the MBG composition [3,4]. In immobilized enzyme systems, the rate-limiting process of the reaction needs to be known for process optimization as well as to understand the fundamental science involved. In addition, the stabilization of enzyme activity over a long period is of significance in achieving higher productivity.

In the present study, we demonstrate esterification catalyzed by lipase in microemulsion-based organogels. The lipase activity is examined as a function of the water and amphiphile concentrations in the gel as they relate to the effectiveness factor. The stability of immobilized lipase is also observed in a repeated batch reaction.

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2. Experimental

2.1. Chemicals

Sodium bis(2-ethylhexyl) sulfosuccinate (AOT, purity 99%) was purchased from Nacalai Tesque (Kyoto). 2,2,4-Trimethyl-pentane (isooctane) was supplied by Wako Pure Chemical Industries (Osaka). Lipase from *Mucor javanicus* (536 units/m g) and gelatin (from porcine skin, Type A, Bloom 300) were obtained from Sigma (St. Louis, MO). Dodecanoic acid and butanol were purchased from Wako. All other chemicals were of analytical reagent grade.

2.2. Preparation of MBGs

The microemulsion phase was prepared by the addition of lipase solution (70 mg solid) to isooctane containing the desired concentration of AOT. Gelatin swelled after the addition of buffer solution. The buffer was composed of 50 mM KH_2PO_4 - 50 mM NaOH solution (pH 7.0). The microemulsion phase and gelatin in buffer were individually heated at 328 K, then vigorously mixed until homogeneous dispersion was achieved. The mixture was then cooled to room temperature. The MBG

thus obtained was stored at 253 K. The water content in the microemulsion phase was determined using a Karl Fisher titrator (MKS-1s; Kyoto Electronics, Kyoto). The water content in the gel phase was calculated from the above preparation procedure.

2.3. *Reaction procedure*

The prepared MBGs (5 cm^3) were cut into cubes of approximately 2 mm unit-length using a razor blade, and then immersed into 10 cm^3 isooctane with magnetic stirring (2 s^{-1}) at 298 K. The reaction was initiated with the addition of 10 cm^3 isooctane solution containing the desired concentrations of dodecanoic acid and butanol. All the reactions were performed at 298 K.

Lipase activity was determined by measuring the production rate of butyl dodecanoate. Butyl dodecanoate in the sample solution was analysed by gas chromatography (GC-8A; Shimadzu, Kyoto) using a glass column packed with FFAP/ Uniport S (60/80 mesh, GL Sciences, Tokyo) and a TCD detector.

3. Results

3.1. *Effect of water and AOT concentrations on reaction rate*

In this study, the W_G (= [H_2O]/[AOT] in the MBG phase) value was used as the MBG water content parameter. Fig. 1 shows the effect of the W_G value on the reaction rate. The reaction rates of this study were determined from the region where ester concentration exhibits a linear increase. The MBG was formed at a W_G value of 80 or above. The reaction rate was found to be maximal at a W_G value of 100. The optimal W_G value corresponded to that of the esterification catalyzed by *Candida rugosa* and *Rhizopus delemar* lipases [3,4].

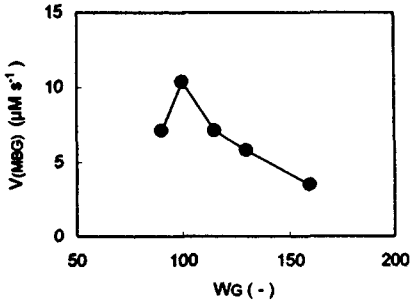

Fig. 1 Effect of W_G value on reaction rate. C_{AOT} = 150 mM; $C_{gelatin}$ = 18% w/v; dodecanoic acid = 100 mM; butanol = 100 mM.

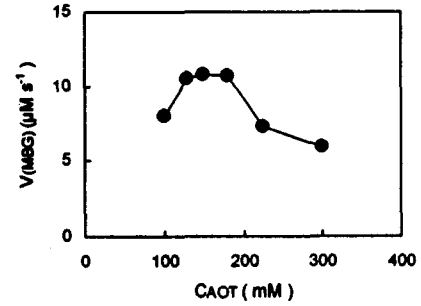

Fig. 2 Effect of AOT concentration on reaction rate. W_G = 100; $C_{gelatin}$ = 18% w/v; dodecanoic acid = 100 mM; butanol = 100 mM.

Fig. 2 shows the effect of AOT concentration in the MBG on the reaction rate. The W_G value was set at 100. The MBG was not formed at an AOT concentration below 80 mM. The reaction rate increased with increasing AOT concentration and reached a maximum at AOT = 150 mM. A similar profile has been reported for the esterification catalyzed by *Candida rugosa* lipase [3].

3.2. *Rate-limiting study*

In an enzyme immobilization system, the enzyme activity is apparently influenced by the mass transfer resistance of substrate. In this study, the influence of the mass transfer

Table 1 Observable modulus, reaction rate and effective diffusion coefficient for dodecanoic acid and butanol as functions of the WG value and the AOT concentration.

WG (-)	Φ^{DA}	Φ^{BTA}	V(MBG) ($\mu M\ s^{-1}$)	Deff^{DA*} ($\times 10^{-10}\ m^2\ s^{-1}$)	Deff^{BTA*} ($\times 10^{-10}\ m^2\ s^{-1}$)
85	0.03	0.05	7.1	2.3	1.7
100	0.04	0.06	10.8	2.5	1.8
115	0.04	0.04	7.1	2.0	1.8
130	0.04	0.04	5.8	1.5	1.8
160	0.04	0.02	3.5	1.0	1.7
CAOT (mM)	Φ^{DA}	Φ^{BTA}	V(MBG) ($\mu M\ s^{-1}$)	Deff^{DA*} ($\times 10^{-10}\ m^2\ s^{-1}$)	Deff^{BTA*} ($\times 10^{-10}\ m^2\ s^{-1}$)
100	0.07	0.05	8.0	1.4	1.8
130	0.08	0.07	10.5	1.5	1.8
150	0.05	0.07	10.8	2.5	1.8
180	0.05	0.07	10.6	2.3	1.7
225	0.04	0.05	7.3	2.3	1.7
300	0.03	0.04	5.9	2.1	1.8

$\Phi = \eta\varphi^2 = V_{(MBG)}\, r_{(MBG)}^2 / 9 D_{eff} C_S$

* *The effective diffusion coefficients of substrates were quarted from Ref.[3] and [5].*

on the overall reaction process was estimated based on the effectiveness factor (η), which is a function of the observable modulus (Φ) as defined by Eq. (1).

$$\Phi = \eta\varphi^2 = \frac{V_{(MBG)}\, r_{(MBG)}^2}{9 D_{eff} C_S} \quad (1)$$

Table 1 shows the observable moduli as a function of the WG value and AOT concentration. As shown in Table 1, the observable moduli within the experimental MBG conditions were in the range of 0.02 - 0.08 for both substrates, giving an effectiveness factor of 1 [6].

3.3. Stability of immobilized lipase activity

Fig. 3 shows the stability of the immobilized lipase activity during in batch reactions. The MBGs used were composed of WG = 100, CAOT = 150 mM and Cgelatin = 18% w/v. As shown in Fig. 3, the lipase activity was maintained for 10 days.

The water molecules, which are formed as a by-product as the reaction progresses were accumulated in the MBGs. This

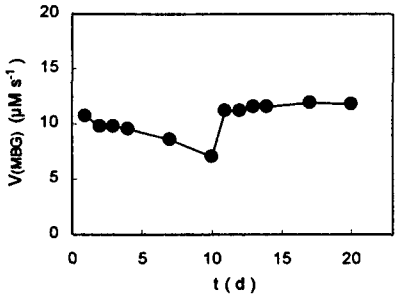

Fig. 3 Stability of immobilized lipase activity in re-use of repeated batch reactions. Dodecanoic acid = 100 mM; butanol = 100 mM.

accumulation of water has been pointed out as one of the main factors for the decrease in the lipase activity [1,3]. Removal of the water accumulated in the MBGs was performed by contacting with 1 M AOT/isooctane solution [1,3]. By this procedure, the lipase activity was recovered to the same level in comparison with the initial MBG used.

4. Discussion

The experimental results in this article prove that the MBG containing *Mucor javanicus* lipase is useful biocatalyst for the esterification in hydrophobic media.

As the lipase activity is affected by the water and amphiphile concentrations, these findings could suggest that the enzymatic characterization of the MBG system is similar to that of the w/o microemulsion system.

Based on the rate-limiting study, the reaction appeared to progress under the reaction-controlled process over the experimental MBG conditions. This result indicates that the change of the lipase activity was caused by alterations in kinetic factors, not by diffusion phenomena in gel particles.

In regard to productivity, the high stability

of the lipase activity was attained by the enzyme immobilization in the MBG. However, the removal of accumulated water from the MBG using a concentrated amphiphile solution needs to be done in typical reaction operation.

5. Conclusions

AOT MBGs entrapping *Mucor javanicus* lipase were investigated as reaction tools for the esterification of dodecanoic acid with butanol in hydrophobic media. The reaction rate was found to be maximal at W_G = 100 and C_{AOT} = 150 mM. Immobilized lipase activity was maintained over 10 days. The loss activity was recovered well by the removal of water accumulated in the MBGs during reaction progresses.

6. List of symbols

C_{AOT}	concentration of AOT in MBG phase (mM)
Cgelatin	concentration of gelatin in MBG phase (% w/v)
Cs	concentration of substrate in bulk organic phase (mM)
Deff	effective diffusion coefficient of substrate in MBG phase ($m^2 s^{-1}$)
$r_{(MBG)}$	radius of MBG particle (mm)
t	time (d)
$V_{(MBG)}$	reaction rate in MBG phase ($\mu M s^{-1}$)
W_G	molar ratio of water to AOT in MBG phase (mol-H2O/mol-AOT)

Greek symbols

η	effectiveness factor (-)
Φ	observable modulus (-)
φ	Thiele modulus (-)

Superscripts

BTA	butanol
DA	dodecanoic acid

References

[1] T.R.J. Jenta, G. Batts, G.D. Rees and B.H. Robinson, Biocatalysis using gelatin microemulsion-based organogels containing immobilized *Chromobacterium viscosum* lipase, Biotechnol. Bioeng., 53 (1997) 121-131.

[2] G.D. Rees, M. G. Nascimento, T.R.J. Jenta and B.H. Robinson, Reverse enzyme synthesis in Microemulsion- based organo-gels, Biochim. Biophys. Acta, 1073 (1991)

[3] K. Nagayama, K. Karaiwa, T. Doi and M. Imai, Esterification activity and stability of *Candida rugosa* lipase in AOT microemulsion-based organogels, Biochem. Eng. J., 2 (1998) 121-126.

[4] K. Nagayama, T. Tada, K. Naoe and M. Imai, *Rhizopus delemar* lipase in microemulsion-based organogels: Reactivity and rate-limiting study, Biocatal. Biotransform., 21 (2003) 321- 324.

[5] K. Nagayama and M. Imai, Effective diffusion and partition coefficients of fatty acids and alcohols in di-2-ethylhexyl sodium sulfosuccinate-gelatin combination microemulson-based organogels, Kagaku Kogaku Ronbunsyu, 29 (2003) 568-571. (in Japanese)

[6] H.W. Blanch and D.S. Clark, Biochemical Engineering, Marcel Dekker Inc, New York, 1997.