KINETICS AND MECHANISM OF FREE AND IMMOBILIZED SULFATASE FROM HELIX POMATIA

HARUO ISHIKAWA, KATSUO KUROSE, MAKOTO OOGAITO AND HARUO HIKITA

Department of Chemical Engineering, University of Osaka Prefecture, Sakai 591

Key Words: Biochemical Engineering, Enzyme Reaction, Separation, Sulfatase, Naphthol, Naphthyl Sulfate, Membrane-Bound Enzyme

As a fundamental study to develop an efficient method for the separation of α- and β-naphthols by combining enzymatic reaction and selective extraction, we investigated the kinetics and the mechanism of the hydrolysis of α- and β-naphthyl sulfates catalyzed by free sulfatase from *Helix pomatia*. These hydrolysis reactions occur competitively and obey a Michaelis–Menten type mechanism. The products, α- and β-naphthols, are inhibitors of both hydrolysis reactions. The enzyme was immobilized in porous gel lattices of albumin and glutaraldehyde. We measured the initial rates of the hydrolysis reactions catalyzed by the membrane-bound sulfatase and analyzed them in terms of the catalytic effectiveness factor. The kinetic parameters evaluated were almost the same as those for free enzyme, except that K_m for α-naphthyl sulfate and k_B/k_A the ratio of the production rate constants were respectively 1/6.1 and 1/2.4 those of free enzyme.

Introduction

Enzymatic reactions have often been utilized for the separation of various isomers such as optical isomers and positional isomers. Recently Pelsy and Klibanov[10] have found that sulfatase from *Helix pomatia* hydrolyzes β-naphthyl sulfate much faster than α-naphthyl sulfate and therefore these reactions can be used for the separation of α- and β-naphthols, which are industrially important positional isomers. In aqueous solutions, both the naphthyl sulfates exist as ionic forms, but the product β-naphthol has a molecular form. Since only the product can dissolve in water-insoluble organic solvents such as decanol and benzene, β-naphthol can be separated by extraction from the unhydrolyzed α- and β-naphthyl sulfates. In a previous paper,[6] we proposed a new separation process which combines effectively the use of an extraction and reactions catalyzed by membrane-bound enzymes. Since there is a good possibility that this process will prove suitable for the practical separation of α- and β-naphthols, the exact mechanism and kinetics of the reactions catalyzed by sulfatase and a method for obtaining a sufficiently active membrane-bound sulfatase needed to be investigated.

We carried out hydrolysis reactions of α- and β-naphthyl sulfates catalyzed by free sulfatase to clarify the reaction mechanism and kinetics. Then we tried several methods for immobilizing sulfatase in membrane. Using prepared membrane-bound sulfatase, the initial rates of the hydrolysis reactions were measured and the kinetic parameters were evaluated.

Received March 7, 1988. Correspondence concerning this article should be addressed to H. Ishikawa.

1. Theoretical

1.1 Kinetics and mechanism of free enzyme

In a solution containing α- and β-naphthyl sulfates, sulfatase catalyzes the following hydrolysis reactions:

$$\alpha\text{-naphthyl sulfate} + H_2O \longrightarrow \alpha\text{-naphthol} + SO_4^{2-} + 2H^+ \quad (1)$$

$$\beta\text{-naphthyl sulfate} + H_2O \longrightarrow \beta\text{-naphthol} + SO_4^{2-} + 2H^+ \quad (2)$$

If we assume that both reactions obey a Michaelis-Menten type mechanism and both products, α- and β-naphthols, are inhibitors of the reactions, the reaction mechanism can be written as follows.

$$S_A + E \rightleftarrows ES_A \longrightarrow P_A + E \quad (3)$$

$$S_B + E \rightleftarrows ES_B \longrightarrow P_B + E \quad (4)$$

$$P_A + E \rightleftarrows EP_A \quad (5)$$

$$P_B + E \rightleftarrows EP_B \quad (6)$$

The rate equations based on the pseudo-steady state assumption can be obtained as

$$-\frac{dS_A}{dt} = \frac{dP_A}{dt} = \frac{V_{mA}S_A}{S_A + K_{mA}(1 + S_B/K_{mB} + P_A/K_{iA} + P_B/K_{iB})} \quad (7)$$

$$-\frac{dS_B}{dt}=\frac{dP_B}{dt}$$

$$=\frac{V_{mB}S_B}{S_B+K_{mB}(1+S_A/K_{mA}+P_A/K_{iA}+P_B/K_{iB})} \quad (8)$$

with six kinetic parameters, V_{mA}, V_{mB}, K_{mA}, K_{mB}, K_{iA} and K_{iB}.

For the reaction solution containing either α-naphthyl sulfate or β-naphthyl sulfate, Eqs. (7) and (8) reduce to Eq. (9).

$$-\frac{dS}{dt}=\frac{dP}{dt}=\frac{V_mS}{S+K_m(1+P/K_i)} \quad (9)$$

In general, the kinetic parameters V_m, K_m and K_i are determined with the initial rates measured at various substrate concentrations. However, in the present work an integration method utilizing the entire reaction curve is used for the determination of the kinetic parameters.

Elimination of P in Eq. (9) with the mass balance equation

$$S_0=S+P \quad (10)$$

and integration of the resultant equation with the initial condition

$$t=0;\ S=S_0,\quad P=0 \quad (11)$$

give

$$\frac{t}{S_0-S}=\frac{K_m(1+S_0/K_i)}{V_m}\cdot\frac{\ln(S_0/S)}{S_0-S}+\frac{1-K_m/K_i}{V_m} \quad (12)$$

The plot of $t/(S_0-S)$ vs. $\ln(S_0/S)/(S_0-S)$ will give a straight line under the assumed reaction mechanism. The slopes of the two straight lines for two different substrate concentrations will differ from each other while these two lines intercept the ordinate at the same point. A set of two slopes and one intercept value is sufficient for the evaluation of three parameters, K_m, K_i and V_m.

The values of K_i and K_m can be determined as

$$K_i=\frac{a''S_0'-a'S_0''}{a'-a''} \quad (13)$$

$$K_m=\frac{1}{(1+a'S_0')/K_i+a'} \quad (14)$$

where a' and a'' express the ratios of the intercept to the slope of the straight lines for the initial substrate concentrations S_0' and S_0'', respectively. The value of V_m can be determined from the intercept b and the K_i and K_m values already determined, as

$$V_m=\frac{1-K_m/K_i}{b} \quad (15)$$

Theoretically, the above-mentioned method can be used to determine the three kinetic parameters for each of the α- and β-isomers. However, as the rate of hydrolysis of α-naphthyl sulfate is very low, as will be shown later, this method introduces considerable error, especially for K_{mA} when applied to α-isomers. In the present work, we used the method discussed below to determine K_{mA} more accurately.

If we assume that the rate of hydrolysis of α-naphthyl sulfate is very low compared with that of β-naphthyl sulfate, Eqs. (8) and (10) can be integrated, assuming that $S_A=S_{A0}=$constant and $P_A=0$, as

$$\frac{t}{S_{B0}-S_B}=\frac{K_{mB}(1+S_{B0}/K_{iB}+S_{A0}/K_{mA})}{V_{mB}}\cdot\frac{\ln(S_{B0}/S_B)}{S_{B0}-S_B}+\frac{1-K_{mB}/K_{iB}}{V_{mB}} \quad (16)$$

According to Eq. (16), the plot of $t/(S_{B0}-S_B)$ vs. $\ln(S_{B0}/S_B)/(S_{B0}-S_B)$ gives a straight line. Therefore, K_{mA} can be determined by

$$K_{mA}=\frac{a^*S_{A0}}{1/K_{mB}-(1+a^*S_{B0})/K_{iB}-a^*} \quad (17)$$

where a^* expresses the ratio of the intercept to the slope of the staight line. The values of K_{iB} and K_{mB} can be determined by Eqs. (13) and (14), respectively.

Once a reliable value of K_{mA} is evaluated by Eq. (17), the values of K_{iA} and V_{mA} can be determined from the experimental reaction data obtained with the reaction mixture containing only α-naphthyl sulfate as a substrate. K_{iA}, the K_i value for α-naphthol, is determined by

$$K_{iA}=\frac{K_{mA}(1+aS_{A0})}{1-aK_{mA}} \quad (18)$$

where a expresses the ratio of the intercept to the slope of the straight line drawn according to Eq. (12). The V_m value for α-naphthyl sulfate, V_{mA}, can be obtained from Eq. (15).

1.2 Kinetics of membrane-bound sulfatase

When a reaction solution containing substrate S is brought into contact with an immobilized enzyme membrane in a batch reactor, the mass balance equation for S can be expressed under the quasi-steady state assumption by

$$-V\frac{dS_b}{dt}=k_LA(S_b-S_s)=AL\eta\frac{V_mS_s}{S_s+K_m^*} \quad (19)$$

where η expresses the effectiveness factor, and the apparent Michaelis constant K_m^* is defined by

$$K_m^*=K_{mB}\left(1+\frac{S_{As}}{K_{mA}}+\frac{P_{As}}{K_{iA}}+\frac{P_{Bs}}{K_{iB}}\right) \quad (20)$$

when the reaction solution contains α-naphthyl sulfate, α-naphthol or β-naphthol in addition to β-naphthyl sulfate, and

$$K_m^*=K_{mA} \quad (21)$$

when the reaction mixture contains only α-naphthyl sulfate as a substrate.

The effectiveness factor η of the membrane-bound enzyme for which the reaction obeys the Michaelis-Menten mechanism is given approximately by[9]

$$\eta=\frac{\eta_0+\alpha\eta_1}{1+\alpha} \tag{22}$$

$$\eta_0=\begin{cases}1 & 0<m\leqq 1\\ 1/m & 1<m\end{cases} \tag{23}$$

$$\eta_1=\tanh m/m \tag{24}$$

$$\alpha=K_m^*/S_S \tag{25}$$

$$m=\frac{L}{1+\alpha}\cdot\sqrt{\frac{V_m}{2D_{eS}S_S}}\cdot\frac{1}{\sqrt{1-\alpha\ln(1+1/\alpha)}} \tag{26}$$

From Eq. (19), the concentration of the substrate S at the membrane surface and the reaction rate R per unit volume of the enzyme membrane are given by

$$S_s=S_b-\left(\frac{V}{k_LA}\cdot\frac{dS_b}{dt}\right) \tag{27}$$

$$R=-\frac{V}{AL}\cdot\frac{dS_b}{dt}=\eta\frac{V_mS_s}{S_s+K_m^*} \tag{28}$$

Eq. (28) can be rewritten as

$$R/\eta=V_m-K_m^*\left(\frac{R/\eta}{S_s}\right) \tag{29}$$

With the initial reaction rates measured at several substrate concentrations, the parameters K_m^* and V_m can be determined by the least-squares method and a trial-and-error procedure by using Eqs. (20) through (29).

The values of K_{mA}, K_{mB}, K_{iA} and K_{iB} can be determined from the K_m^* values measured by using the initial rate data obtained with various reaction solutions containing (1) only β-naphthyl sulfate, (2) β-naphthyl sulfate and α-naphthol, (3) β-naphthyl sulfate and β-naphthol, (4) α- and β-naphthyl sulfates, and (5) only α-naphthyl sulfate.

2. Experimental Apparatus and Procedure

2.1 Materials

The sulfatase from *H. pomatia* was purchased from Sigma (St. Louis, Mo). α- and β-naphthols, buffer solution compounds, HCl, and other chemicals were of high purity and were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan).

α- and β-naphthyl sulfates were both synthesized in our laboratory according to the method of Pelsy and Klibanov.[10]

2.2 Measurement of rates of reactions catalyzed by free enzyme

The reactor was a 10-ml vial with water jacket in which temperature-regulated water was continuously circulated at 37°C. In the reactor 5.0 ml of 0.1 kmol/m^3 phosphate buffer (I = 0.3 kmol/m^3) containing a single substrate or two substrates was stirred with a Teflon-coated magnetic stirrer bar at 500 rpm. After the liquid temperature reached 37°C, 100 μl of an enzyme solution was injected into the reaction solution to start the reaction. Liquid samples of 200 μl each were taken at appropriate time intervals for 3 h. The samples taken were mixed at once with an equal volume of 0.1 kmol/m^3 phosphoric acid of which pH was adjusted to 2 by using KOH to stop the reaction and were analyzed for α- and β-naphthols by a Shimadzu-LC-5 liquid chromatography with a Zorbax BP-ODS column and a spectrophotometric detector at a wavelength of 286 nm.

Table 1. Experimental conditions of reactions catalyzed by free sulfatase

Run	S_{A0} [kmol/m^3]	S_{B0} [kmol/m^3]	E_0 [kg/m^3]
F-1	0	2.47×10^{-3}	0.14
F-2	0	4.94×10^{-3}	0.14
F-3	8.61×10^{-3}	4.94×10^{-3}	0.14
F-4	1.41×10^{-2}	4.94×10^{-3}	0.14
F-5	2.71×10^{-2}	0	1.5

The other experimental conditions are summarized in **Table 1**.

2.3 Immobilization of enzyme

Sulfatase was immobilized on porous glass by diazo-coupling,[13] on concavanadin A by physical adsorption,[1] and on activated alumina by covalent bonding.[10] Since these methods cannot be used for preparing a membrane-bound sulfatase, however, we tried several other immobilization methods such as covalent bonding to cyanogen bromide-activated cellulose membranes[2] or alkylated cellulose membranes,[12] ionic bonding to CM-cellulose or DEAE-cellulose,[8] and entrapment in gel lattices of polyacrylamide[3] or albumin cross-linked by glutaraldehyde.[11] Of these only the entrapment method produced a catalytic membrane with sulfatase activity.

The immobilization procedure was as follows. (1) Dissolve bovine serum albumin and sulfatase in 0.01 kmol/m^3 phosphate buffer of pH 7.8 to concentrations 10–15 wt% and 13–150 kg/m^3, respectively. (2) Add glutaraldehyde to this solution to a concentration of about 0.5 vol%. (3) Stir the solution quickly and pour it onto a clean glass plate. (4) Place the support membrane (Millipore RATF, material: mixed esters of cellulose, pore size: 1.2 μm, thickness: 145 μm, porosity: 82.0%) on the solution and let the solution permeate uniformly in the membrane. Leave it in the air for about 30 min for gelation, and then immerse it into a 1.0 vol% glutaraldehyde solution for about 2 h for further hardening of the membrane. (5)

Table 2. Experimental conditions and results of initial rates of hydrolysis of β-naphthyl sulfate catalyzed by membrane-bound sulfatase at pH 7.8 and 37°C

Run	S_{A0} [kmol/m³]	S_{B0} [kmol/m³]	P_{A0} [kmol/m³]	P_{B0} [kmol/m³]	v_{PB} [kmol/m³ s]
M-1	0	4.27×10^{-3}	0	0	1.64×10^{-7}
M-2	0	5.61×10^{-3}	0	0	1.85×10^{-7}
M-3	0	6.91×10^{-3}	0	0	2.09×10^{-7}
M-4	0	8.34×10^{-3}	0	0	2.20×10^{-7}
M-5	0	9.56×10^{-3}	0	0	2.33×10^{-7}
M-6	0	1.83×10^{-3}	5.62×10^{-3}	0	6.16×10^{-8}
M-7	0	3.32×10^{-3}	5.62×10^{-3}	0	1.00×10^{-7}
M-8	0	4.81×10^{-3}	5.62×10^{-3}	0	1.30×10^{-7}
M-9	0	6.51×10^{-3}	5.62×10^{-3}	0	1.57×10^{-7}
M-10	0	6.48×10^{-3}	0	3.90×10^{-3}	1.09×10^{-7}
M-11	0	7.50×10^{-3}	0	3.90×10^{-3}	1.26×10^{-7}
M-12	0	1.02×10^{-2}	0	3.90×10^{-3}	1.48×10^{-7}
M-13	0	1.17×10^{-2}	0	3.90×10^{-3}	1.63×10^{-7}
M-14	1.05×10^{-2}	3.75×10^{-3}	0	0	7.50×10^{-8}
M-15	1.05×10^{-2}	4.98×10^{-3}	0	0	1.02×10^{-7}
M-16	1.05×10^{-2}	6.75×10^{-3}	0	0	1.14×10^{-7}
M-17	1.05×10^{-2}	9.48×10^{-3}	0	0	1.57×10^{-7}
M-18	1.05×10^{-2}	0	0	0	1.38×10^{-8}*

* v_{PA}.

Wash the membrane well with distilled water and store it in a 0.1 kmol/m³ phosphate buffer (pH 7.8) at about 4°C.

2.4 Characteristics of membrane-bound sulfatase

The effects of the concentrations of albumin and enzyme on the activity of the immobilized enzyme were studied by measuring the initial overall rates of the hydrolysis reaction by using apparatus and procedure similar to those used in 2.5 below.

The diffusivities of β-naphthol in the catalytic membranes were measured by using apparatus and procedure similar to those used by Ishikawa *et al.*[5]

2.5 Measurement of rates of reactions catalyzed by membrane-bound sulfatase

Figure 1 shows the experimental apparatus used for measuring the initial reaction rates catalyzed by the membrane-bound sulfatase. A catalytic membrane was placed directly on the gasket set on the end plate and the periphery of the membrane was held in place by a doughnut-shaped plate. The effective membrane surface area was made equal to the open area of the plate, 7.06 cm³.

The liquid temperature was kept constant at 37°C by circulating temperature-regulated water in the water jackets. In the reactor, 37.7 ml of 0.1 kmol/m³ phosphate buffer containing the substrate was injected at 37°C and immediately the reactor was stirred with a Teflon-coated magnetic bar at 500 rpm. Liquid samples of 100 μl each were taken at appropriate time intervals for 30 min. Each sample was diluted with 1.0 ml of water and then analyzed by liquid chromatography for α- and β-naphthols as mentioned in 2.2. The concentrations of α- and β-naphthyl sulfates were also determined by liquid chromatography after they were hydrolyzed with HCl.

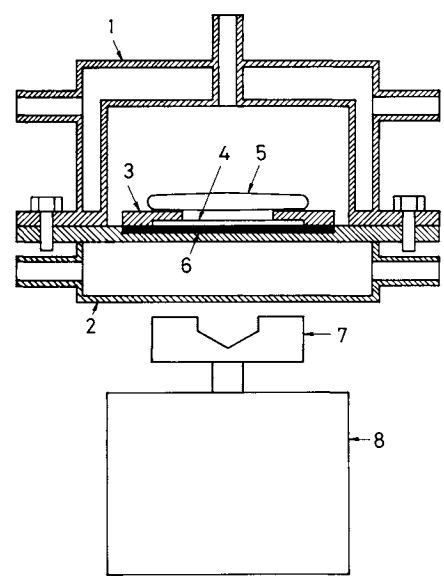

Fig. 1. Experimental apparatus used for measuring initial reaction rates catalyzed by membrane-bound sulfatase
1), 2) water jacket; 3) cover plate; 4) catalytic membrane; 5) magnetic stirring bar; 6) gasket; 7) magnet; 8) motor

Other experimental conditions are given in **Table 2.**

3. Results and Discussion

3.1 Kinetics and mechanism of free enzyme

Figure 2 shows the experimental initial rates r_{PB} of the hydrolysis of β-naphthyl sulfate and the ratio r_{PB}/r_{PA} of the initial rates as a function of pH of the solution. As can be seen in the figure, the rates of the

hydrolysis of α-naphthyl sulfate are quite low compared with those of β-naphthyl sulfate. Both the r_{PB} and r_{PB}/r_{PA} values become maximum at pH 7.8. These results are consistent with those of Pelsy and Klibanov.[10] The following investigations into the reaction mechanism and the kinetics were carried out at pH 7.8.

Figure 3 shows experimental reaction curves in which product concentrations are plotted against time. The rate of production of β-naphthol in Run F-2 is almost twice that in Run F-1. Comparison of the results of Runs F-2 through F-4 shows that the existence of α-naphthyl sulfate in the reaction solution decreases the hydrolysis rate of β-naphthyl sulfate, and that the higher the α-naphthyl sulfate concentration, the lower is the production rate of β-naphthol. This means that α-naphthyl sulfate inhibits the hydrolysis of β-naphthyl sulfate catalyzed by sulfatase. Furthermore, Fig. 3 shows that the amount of α-naphthol produced in Run F-5 is similar to that of β-naphthol produced in Run F-1. This means that the hydrolysis rate of α-naphthyl sulfate is much less than that of β-naphthyl sulfate, because the enzyme concentration in Run F-5 is 10.7 times that in Run F-1.

Figure 4 shows replots of the experimental results of Runs F-1, F-2 and F-4 shown in Fig. 3. **Figure 5** shows a replot of results from Run F-5, also shown in Fig. 3. The plots of $t/(S_0-S)$ vs. $\ln(S_0/S)/(S_0-S)$ correlate the experimental results well with straight lines for both the hydrolysis reactions of the α- and β-naphthyl sulfates, indicating that the reactions obey the mechanism postulated in 1.1. The slopes and the intercepts of the correlation lines in Figs. 4 and 5 were analyzed by the methods described in 1.1 to determine the kinetic parameters. The results are tabulated in **Table 3** where the K_{mB} value of Pelsy and Klibanov[10] is also shown for comparison. The K_{mB} value reported by them agrees well with that of the present work while the K_{mA} value was not examined due to lack of reference data. The experimental time courses of Runs F-1, F-2, F-4 and F-5 were used to evaluate the kinetic parameters with which the individual solid lines in Fig. 3 were calculated using the corresponding rate equations discussed in 1.1. The experimental results of Runs F-1, F-2, F-4 and F-5 are well reproduced by the solid lines shown in the figure. It is also shown that the experimental results of Run F-3 agree well with the theoretical predictions although these data were not used for evaluating the kinetic parameters.

From these results, we consider that the reaction mechanisms assumed in 1.1 are reasonable and the determined kinetic parameters are sufficiently accurate.

3.2 Characteristics of catalytic membranes

The overall reaction rate of hydrolysis increased

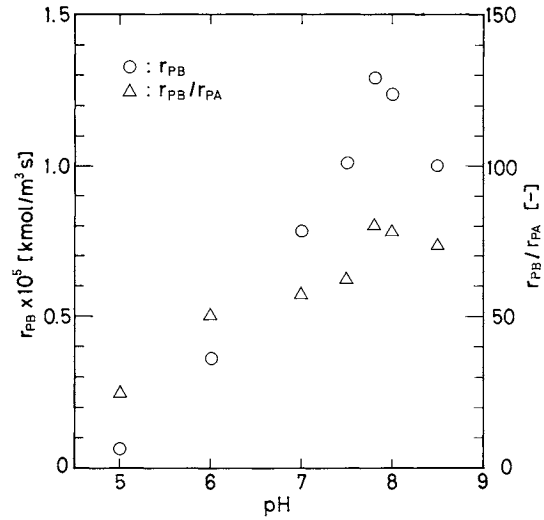

Fig. 2. Effect of pH on initial rates of hydrolysis of α- and β-naphthyl sulfates catalyzed by free sulfatase

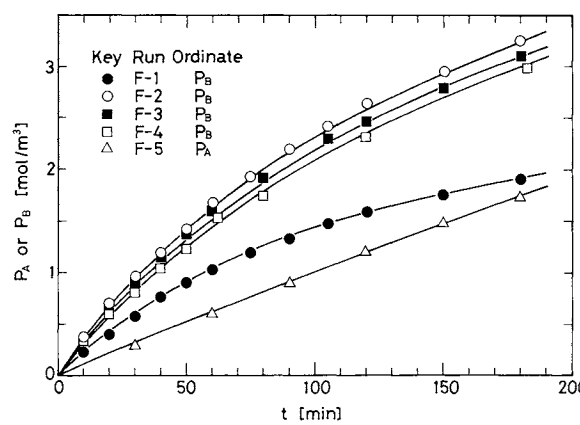

Fig. 3. Reaction curves for hydrolysis of α- and β-naphthyl sulfates catalyzed by free sulfatase

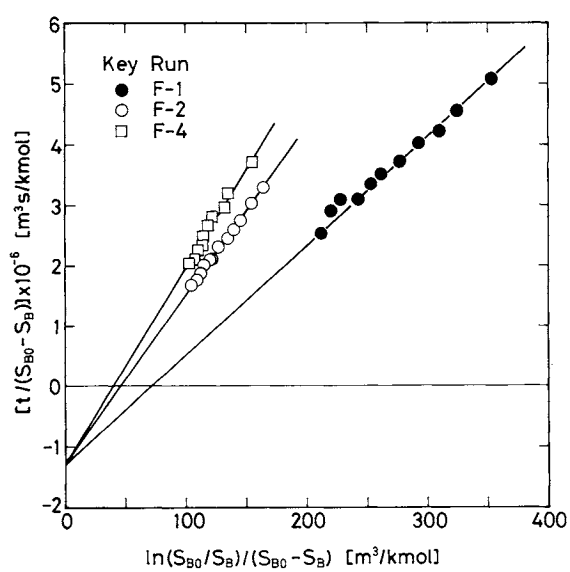

Fig. 4. Experimental results of hydrolysis of β-naphthyl sulfates catalyzed by free sulfatase. Plot of $t/(S_{B0}-S_B)$ vs. $\ln(S_{B0}/S_B)/(S_{B0}-S_B)$

Table 3. Determined kinetic parameters for free and membrane-bound sulfatase at pH 7.8 and 37°C

	K_{mA} [kmol/m^3]	K_{mB} [kmol/m^3]	K_{iA} [kmol/m^3]	K_{iB} [kmol/m^3]	k_B/k_A [—]
Free enzyme					
Present work	3.02×10^{-2}	5.24×10^{-3}	7.01×10^{-3}	1.97×10^{-3}	38.4
Pelsy and Klivanov's work	—	4.50×10^{-3}	—	—	—
Membrane-bound enzyme					
Present work	4.96×10^{-3}	3.31×10^{-3}	7.78×10^{-3}	1.47×10^{-3}	15.9

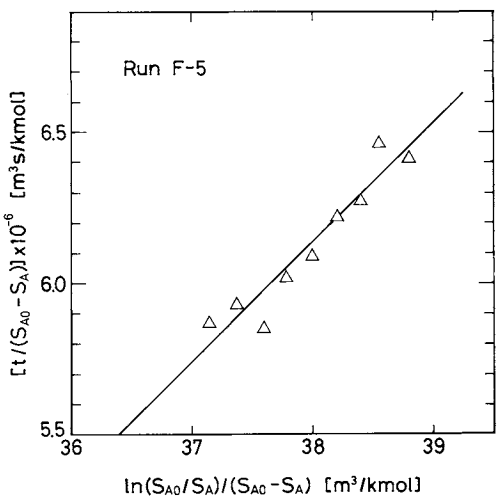

Fig. 5. Experimental results of hydrolysis of α-naphthyl sulfate catalyzed by free sulfatase. Plot of $t/(S_{A0}-S_A)$ vs. $\ln(S_{A0}/S_A)/(S_{A0}-S_A)$

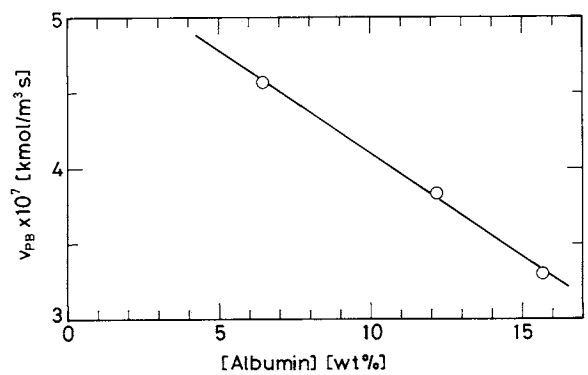

Fig. 6. Effect of albumin concentration on initial rates of hydrolysis of β-naphthyl sulfates catalyzed by membrane-bound sulfatase

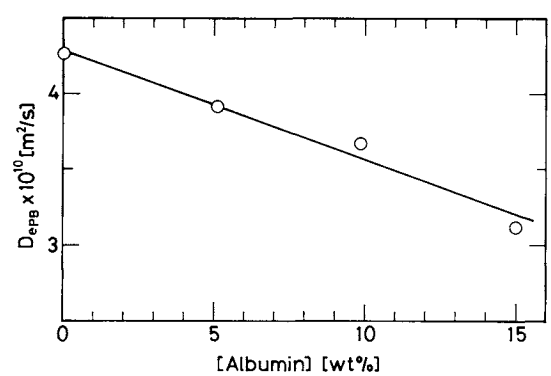

Fig. 7. Effect of albumin concentration on effective diffusivities of β-naphthol in catalytic membrane

with the amount of enzyme used for immobilization. When the enzyme concentration exceeded 150 kg/m^3, obtaining a catalytic membrane in which the enzyme was distributed uniformly was difficult due to the rapid polymerization reaction of albumin.

Figure 6 shows that the rate decreases with albumin concentration, and that for higher activity a lower albumin concentration is favorable. On the other hand, a low albumin concentration does not give sufficient mechanical stability of the membrane. For preparing membrane-bound sulfatase with a higher enzyme activity and tolerable mechanical stability, we determined the enzyme and albumin concentrations to be 100–140 kg/m^3 and 12–14 wt%, respectively.

Figure 7 shows the effective diffusivities of β-naphthol in the catalytic membrane. Effective diffusivity decreases with concentration of albumin used. From the effective diffusivity D_{ePB} of β-naphthol at zero albumin concentration, the diffusivity D_{PB} of β-naphthol in 0.1 kmol/m^3 phosphate buffer (pH 7.8) can be evaluated by

$$D_{PB}=\frac{\tau D_{ePB}}{\varepsilon} \tag{30}$$

where ε expresses the porosity and τ the tortuosity. The τ value was evaluated as 3.1 by a method similar to that in our previous work.[5] The determined diffusivity of β-naphthol in 0.1 kmol/m^3 phosphate buffer was 1.61×10^{-9} m^2/s at 37°C. The diffusivity of β-naphthyl sulfate was evaluated from that of β-naphthol as 1.29×10^{-9} m^2/s at 37°C on the basis that $D\propto$(molecular weight)$^{-1/2}$.[7] In this estimation the effect of albumin concentration on the diffusivity of β-naphthyl sulfate was assumed to be the same as that on the diffusivity of β-naphthol.

The diffusivities of α-naphthol and α-naphthyl sulfate were assumed to be equal to those of β-naphthol and β-naphthyl sulfate, respectively.

3.3 Kinetics of membrane-bound enzyme

Figure 8 shows the initial hydrolysis rate v_{PB} of β-naphthyl sulfate and the ratio v_{PB}/v_{PA} of the initial hydrolysis rates as a function of pH of the solution. Both the rate and the ratio become maximum at pH 7.8. The comparison of Fig. 8 with Fig. 2 shows that

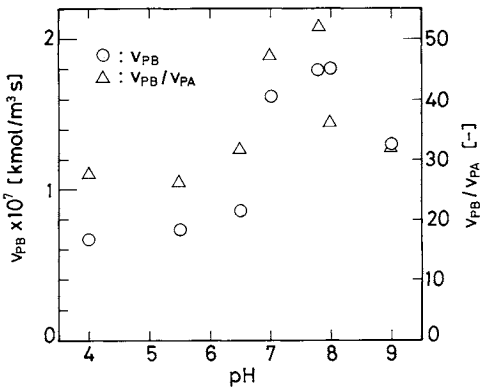

Fig. 8. Effect of pH on initial rates of hydrolysis of α- and β-naphthyl sulfates catalyzed by membrane-bound sulfatase

the hydrolysis rate and the ratio of the hydrolysis rates for the membrane-bound sulfatase become maximum at the same pH as that for free sulfatase. All the following experimental results were obtained at pH 7.8.

The initial hydrolysis rates are given in Table 2 with their experimental conditions. These results were analyzed by the procedure described in 1.2. The k_L value required for analysis is 8.06×10^{-5} m/s at 500 rpm, which was determined by measuring the rate of dissolution of benzoic acid in the same apparatus used for measurements of the reaction rates catalyzed by a membrane-bound enzyme. The values of V_{mB} and K_{mB} were determined from the results of Runs M-1 through M-5. The values of K_{iA}, K_{iB}, K_{mA} and V_{mA} were determined from the results of Runs M-6 through M-9, M-10 through M-13, M-14 through M-17, and M-18, respectively, in a consecutive manner. The determined kinetic parameters are given in Table 3 in parallel with those for the free enzyme. The values of K_{mB}, K_{iA} and K_{iB} for the membrane-bound sulfatase are almost the same as those for the free sulfatase. However, the values of K_{mA} and k_B/k_A for the membrane-bound sulfatase are about 1/6.1 and 1/2.4 of the values for the free sulfatase, respectively. Both of these results are responsible for the fact that the relative hydrolysis rate of α-naphthyl sulfate to that of β-naphthyl sulfate increased when the reaction was catalyzed by immobilized sulfatase. It was observed often[4] that the K_m values of enzyme reactions varied when enzymes were immobilized. The change in K_m values was attributed to the change of the microenvironment around the enzyme, the conformation change of the enzyme and sometimes the diffusional effects. On the other hand, almost no attention has been paid to the change in value of k due to immobilization. This is because evaluation of the amount of active enzyme in the immobilized enzyme is very difficult. The finding that the k_B/k_A value for the immobilized enzyme is quite different from that of the free enzyme is very interesting. The explanations for the change in K_m value may also be applicable to this finding. We consider that intensive investigations should be made to explain why and how the k values change due to immobilization.

In the present work, only the membrane-bound sulfatase prepared by trapping enzyme in gel lattices of albumin cross-linked by glutaraldehyde had a considerable activity. By immobilization of enzyme, the K_m value for α-naphthyl sulfate decreased considerably and the hydrolysis rate of α-naphthyl sulfate increased. It is desirable to develop a method of preparing membrane-bound sulfatase with a higher activity for β-naphthyl sulfate and a lower activity for α-naphthyl sulfate.

Nomenclature

A	=	surface area of catalytic membrane	[m²]
a	=	ratio of intercept to slope	[m³/kmol]
b	=	intercept	[m³·s/kmol]
D	=	diffusivity	[m²/s]
D_e	=	effective diffusivity in membrane	[m²/s]
E	=	concentration of enzyme	[kmol/m³]
K_i	=	inhibition constant	[kmol/m³]
K_m	=	Michaelis constant	[kmol/m³]
K_m^*	=	apparent Michaelis constant defined by Eq. (20) or (21)	[kmol/m³]
k	=	rate constant of step ES→P+E, V_m/E_0	[s⁻¹]
k_L	=	mass transfer coefficient	[m/s]
L	=	thickness of catalytic or support membrane	[m]
m	=	parameter defined by Eq. (26)	[—]
P	=	concentration of product	[kmol/m³]
R	=	reaction rate per unit volume of catalytic membrane	[kmol/m³·s]
r	=	rate of reaction catalyzed by free enzyme per unit liquid volume	[kmol/m³·s]
S	=	concentration of substrate	[kmol/m³]
t	=	time	[s]
V	=	liquid volume in reactor	[m³]
V_m	=	maximum reaction rate	[kmol/m³·s]
v	=	rate of reaction catalyzed by membrane-bound enzyme per unit liquid volume	[kmol/m³·s]
α	=	parameter defined by Eq. (25)	[—]
ε	=	porosity of support membrane	[—]
η	=	effectiveness factor	[—]
τ	=	tortuosity of support membrane	[—]

〈Subscripts〉

A	=	α-isomers
B	=	β-isomers
b	=	liquid bulk
s	=	surface of catalytic membrane
0	=	initial

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BUBBLE FORMATION AT A SUBMERGED ORIFICE IN NON-NEWTONIAN AND HIGHLY VISCOUS NEWTONIAN LIQUIDS

TOSHIRO MIYAHARA, WEI-HONG WANG AND TERUO TAKAHASHI
Department of Applied Chemistry, Okayama University, Okayama 700

Key Words: Fluid Mechanics, Bubble Formation, Single Orifice, Non-Newtonian Liquid, Viscous Newtonian Liquid, Biochemical Engineering, Absorption

Bubble formation at a submerged orifice was studied experimentally for non-Newtonian liquids (aqueous CMC solutions) and highly viscous Newtonian liquids (aqueous glycerol solutions). The bubble volumes formed in both non-Newtonian and highly viscous Newtonian liquids are large compared to those formed in relatively low-viscosity Newtonian liquids. The formation regime of the constant-pressure condition, usually found for low-viscosity Newtonian liquids as reported by Tadaki *et al.*,[12] was not observed. Using an extended two-stage bubble-formation model, the bubble volumes formed at a submerged orifice in non-Newtonian and highly viscous Newtonian liquids are considered. The predicted bubble volumes compare satisfactorily with the experimental data over a wide range of parameters of power law liquid ($m <$ around 8 Pa·s^n, $0.95 > n > 0.57$) and viscosities of Newtonian liquids ($5.05\ \text{Pa·s} > \mu_l > 0.439\ \text{Pa·s}$) including the results of the present work and that of Tsuge *et al.*[13,15]

Introduction

Gas-liquid contacting operations such as fermentation, aerobic waste water treatment and polymer production, where most liquids are non-Newtonian and/or highly viscous Newtonian, include processes where gas is dispersed as bubbles through a liquid. However, the phenomena of bubble formation are complex and are not fully described to date. Although bubble formation in relatively low-viscosity Newtonian liquids has been studied extensively,[7] little attention has been paid to bubble formation in non-Newtonian and/or highly viscous Newtonian liquids.

Costes *et al.*[4-5] reported that in aqueous CMC solutions bubbles are formed under constant-pressure conditions for Reynolds numbers lower than 1000, while for Reynolds numbers greater than 1000 they are formed under constant-flow rate conditions. They also noted that a comparison of theoretical variation of bubble radius with the measurements seems to show that the models of Davidson *et al.*[6] and Kumar *et al.*[7] are in good agreement, but that the former is more suitable than the latter for representing bubble formation. On the other hand, Acharya *et al.*[1] compared the data for power-law liquids with those computed using the model of bubble-formation in inviscid liquid and concluded that the rheology of the ambient liquid has no influence on bubble volumes. Rabiger *et al.*[11] proved that single or double bubbles are always formed at the orifice over a wide range of gas throughput in both Newtonian and non-Newtonian liquids. Tsuge *et al.*[13,15-16] recently investigated the effect of various factors on the volume of bubbles formed in highly viscous Newtonian and non-Newtonian liquids in the presence of pressure change in the gas chamber and obtained dimensionless equations for predicting bubble volumes. Thus, in spite of its practical importance, the mechanism of

Received March 36, 1988. Correspondence concerning this article should be addressed to T. Miyahara.