Fibrous Support for Immobilization of Enzymes. II

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Synopsis

A series of adsorption experiments was conducted with varying initial enzyme concentrations. The observed values are compared with the results calculated from the adsorption equations based on Langmuir adsorption mechanism. The values of three parameters, adsorption equilibrium constant K_a, maximum value of adsorbed enzyme per unit mass of SFF, N, and forward adsorption rate constant k_a, were determined from Langmuir adsorption equations and experimental data. In the initial enzyme concentration range below 100 (mg enz./dL), the simulated enzyme concentration vs. time curve agreed well with observed values. However, it was found that the adsorption in the higher concentration range was slow, and the corrected forward adsorption rate constant should be employed.

INTRODUCTION

As described in the previous paper,[1] superfine fiber (SFF) contains the following features and has been found to be an excellent support for immobilizing enzymes:

(1) It is easy for SFF to incorporate various functional groups by acetalization.

(2) SFF is able to adsorb a large amount of enzyme because of its large surface area.

(3) The fibrous support like SFF permits a variety of fibrous shapes suitable for many kinds of enzyme reactors.

(4) The experimental procedure for immobilization is very simple because SFF immobilizes enzymes by ionic bonds.

Many papers have been published on enzymes ionically bound to supports.[2-6] However, little is known about the adsorption rate of enzymes. Therefore, it is necessary to investigate the enzyme adsorption rate in detail in order to clarify the adsorption mechanism and find the optimum condition for immobilizing enzymes. In this study, the adsorption rate of invertase onto dimethyl-aminated SFF[1] was measured and then compared with the results calculated from the simple equations based on the Langmuir adsorption mechanism.

EXPERIMENTAL

According to the method described in the previous paper,[1] dimethyl-aminated SFF samples were left in hot water at 80°C for an hour and then they were immersed overnight in 1*N* HCl under agitation. After pretreatment described above, SFF samples were freeze-dried by freeze-dryer, Freeze-mobile 2 of Virtis and weighed.

Invertase of Seikagaku-Kogyo derived from candida utilis was dissolved in

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50 mL of distilled water. About 0.1 g of freeze-dried SFF was added to the enzyme solution at 37°C and the mixture was then vigorously stirred. The absorbances of the enzyme solution were measured at given time intervals.

In order to lessen the influence of a slight amount of impurities,[7] the absorbances at 285 nm and 295 nm were measured by Hitachi-200 Spectrophotometer (see Fig. 1) and the absorbance difference was related to the enzyme concentration.

After adsorption equilibrium was established, the SFF–invertase conjugate was washed with distilled water and then freeze-dried and weighed.

BASIC EXPRESSIONS

For a solid surface that has uniform properties and therefore holds adsorbed molecules as tightly as all the others present, the equilibrium between adsorbed molecules and gas molecules can be found to follow Langmuir adsorption isotherm which equates the rate of capture of molecules from the gas to the rate of escape of molecules from the surface.

Langmuir isotherms are sometimes applied to liquid–solid equilibria including ion exchange as well.[8]

Giles et al. stated that isotherms for adsorption of organic solutes were divided into four main classes including Langmuir isotherm, according to the nature of slope of the initial portion of the curve.[9] The data obtained from the adsorption experiment of invertase on dimethyl-aminated SFF were plotted in Figure 2.

The curve in Figure 2 has the shape characteristic of Langmuir adsorption

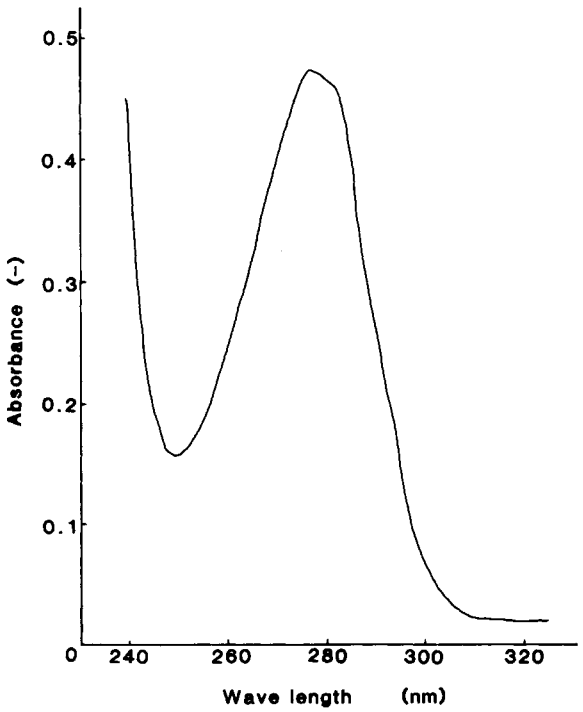

Fig. 1. UV spectrum of invertase: invertase concn = 65 mg enz./dL.

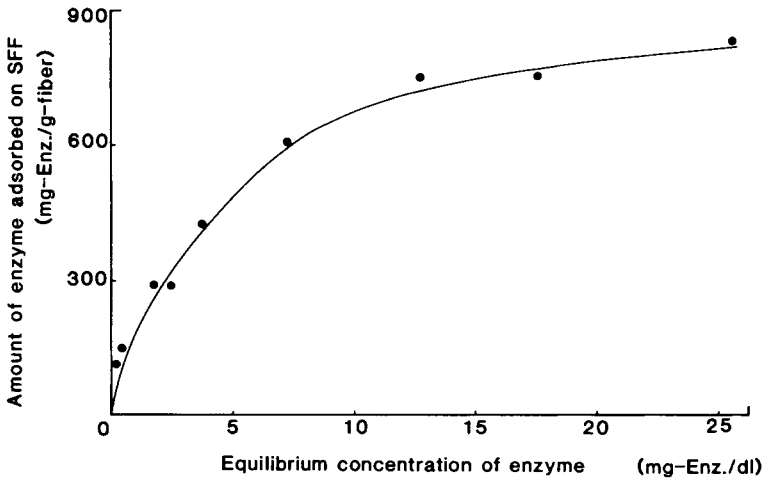

Fig. 2. Adsorption isotherm.

isotherms. For adsorption of a single component obeying a Langmuir isotherm, the rate R_a is

$$R_a = k_a C(1 - \theta) - k_d \theta \tag{1}$$

where R_a = adsorption rate (mg enz./min·g fiber), C = enzyme concentration in solution (mg·enz./dL), θ = fraction of surface covered by adsorbed enzymes, k_a = forward adsorption rate constant (dL/min·g fiber), and k_d = reverse adsorption rate constant (mg enz./min·g fiber). The fraction of the area that is covered is represented by

$$\theta = V(C_0 - C)/NW \tag{2}$$

where V = volume of enzyme solution (dL), C_0 = initial enzyme concentration (mg enz./dL), N = maximum value of adsorbed enzyme per unit mass of SFF (mg enz./g fiber), and W = weight of SFF (g fiber).

At equilibrium condition, eq. (1) is equal to zero and eq. (2) becomes

$$\theta_\infty = K_a C_\infty/(1 + K_a C_\infty) = V(C_0 - C_\infty)/NW \tag{3}$$

where $K_a = k_a/k_d$ = adsorption equilibrium constant (dL/mg enz.) and C_∞ = enzyme concentration at equilibrium (mg enz./dL). Eq. (3) may be rewritten as

$$\frac{W}{V}\frac{C_\infty}{C_0 - C_\infty} = \frac{1}{K_a N} + \frac{1}{N} C_\infty \tag{4}$$

and a plot of $(W/V)\, C_\infty/C_0 - C_\infty$ against C_∞ will give a straight line of slope $1/N$ and intercept $1/K_aN$ on the $(W/V)\, C_\infty/C_0 - C_\infty$ axis. K_a and N can therefore be calculated from the slope and the intercept of the plot.

Assuming that agitation is strong enough to mix enzyme solution and a SFF sample completely, an adsorption rate equation is given by

$$-\frac{V}{W}\frac{dC}{dt} = k_a C(1 - \theta) - k_d \theta \tag{5}$$

Substitution of eq. (2) into eq. (5) gives

$$N\frac{d\theta}{dt} = k_a\left(C_0 - \frac{NW}{V}\theta\right)(1-\theta) - k_d\theta$$
$$= k_a\left[\frac{NW}{V}\theta^2 - \left(\frac{NW}{V} + C_0 + \frac{1}{K_a}\right)\theta + C_0\right] \quad (6)$$

where t = elapsed time (min).

Rearranging eq. (6) yields the following equation:

$$\frac{d\theta}{(NW/V)\theta^2 - (NW/V + C_0 + 1/K_a)\theta + C_0} = \frac{k_a}{N}dt \quad (7)$$

IC at $t = 0, \theta = 0$.

Equation (7) is integrated to give[10]

$$\frac{1}{\sqrt{q^2-4pr}}\log\left|\frac{(2p\theta + q - \sqrt{q^2-4pr})(q+\sqrt{q^2-4pr})}{(2p\theta + q + \sqrt{q^2-4pr})(q-\sqrt{q^2-4pr})}\right| = \frac{k_a}{N}t \quad (8)$$

in which $p = NW/V$, $q = -(NW/V + C_0 + 1/K_a)$ and $r = C_0$. Let the quantity on the left of eq. (8) be $f(\theta)$. Equation (8) suggests a linear relationship between $f(\theta)$ and elapsed time t. k_a should be determined by plotting $f(\theta)$ against time t.

If the theoretical curve calculated from eq. (5) with previously determined k_a and K_a fits well to the experimental data, the adorption model based on the Langmuir mechanism would be reasonable.

RESULTS AND DISCUSSION

The Measurement of Adsorbed Invertase

In our previous paper,[1] the activity of invertase solution was measured before and after an adsorption experiment, and the activity difference was taken as the amount of invertase adsorbed on SFF. However, the experimental procedure is too time-consuming to analyze many samples and also has a problem concerning deactivation. In this work, the absorbance of invertase solution was measured before and after an adsorption experiment, and then the adsorbed invertase was calculated from those data and the calibration curve in Figure 3. Figure 3 shows the plot of the absorbance difference between at 285 and 295 nm against invertase concentration. A quite linear relationship does exist between them.

Two freeze-dried SFF samples (see Table I) were weighed and then used for an adsorption experiment. After the experiment, the SFF–invertase conjugates were freeze-dried and weighed. The weight differences between freeze-dried SFF and SFF–invertase conjugate were 80 mg for sample A and 62 mg for sample B, respectively. On the other side, the amount of the adsorbed invertase obtained from absorbance data were 84 mg for A and 62 mg for B. The former values agreed well with the latters. The procedure, which determined the amount of adsorbed invertase by measuring absorbance differences of the enzyme solution, proved very useful.

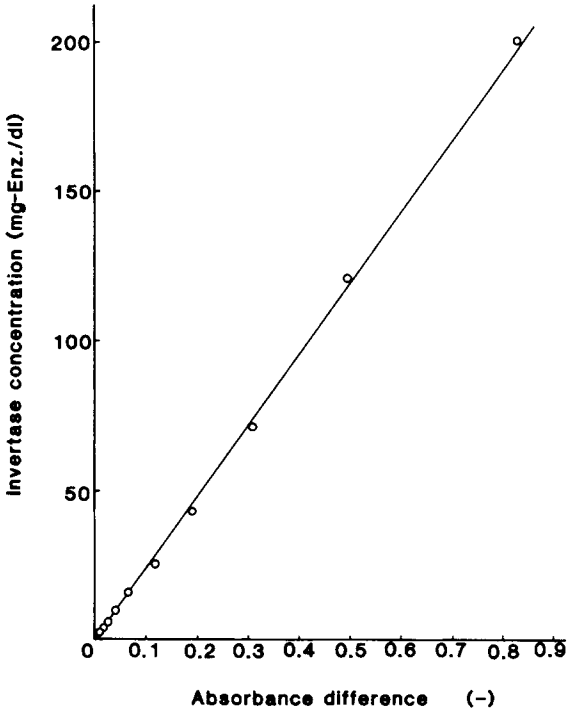

Fig. 3. Calibration curve for absorbance difference and invertase concentration.

The Determination of K_a, N, and k_a

The values of the initial enzyme concentration C_0, the equilibrium concentration C_∞, the SFF weight W, and the solution volume V were substituted into eq. (4) and $(W/V)\ C_\infty/(C_0 - C_\infty)$ was then plotted againsnt C_∞. As shown in Figure 4, the relationship between them is found to be linear. From the slope and the intercept of the straight line, the adsorption equilibrium constant K_a and the weight of adsorbed enzyme corresponding to complete coverage of the SFF surface N were determined to be 0.284 dL/mg enz. and 925 mg enz./g fiber, respectively.

The value of $f(\theta)$ was calculated from eqs. (2) and (8) and the enzyme concentration vs. time curve obtained from the adsorption experiment. $f(\theta)$ was

TABLE I
Amount of Invertase Adsorbed on SFF

Sample	A	B
Invertase (mg)[a]	102	71
SFF (mg)	107	103
SFF with adsorbed invertase (mg)	187	165
Absorbance difference before adsorption experiment	0.846	0.591
Absorbance difference after adsorption experiment	0.145	0.072

[a] The figures in this line represent the amounts of invertase added in the each adsorption experiment.

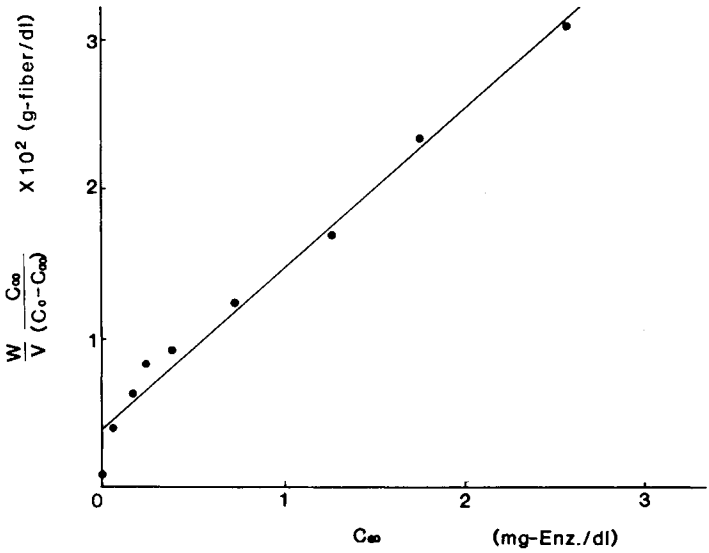

Fig. 4. Plots to determine N and K_a.

plotted against elapsed time t, and then the forward adsorption rate constant k_a was calculated from the slope. When enzyme concentration is relatively low, enzymes are adsorbed on the apparent SFF surface, which means a part of SFF directly exposed to the bulk enzyme solution, and the adsorption process seems to follow the Langmuir adsorption mechanism. When the initial enzyme concentration is less than 100 mg enz./dL, a plot of $f(\theta)$ vs. t gives a straight line and then the forward adsorption rate constant k_a is calculated at 0.647 dL/min·g fiber from the slope (see Fig. 5). However, as an adsorption proceeds and most of the

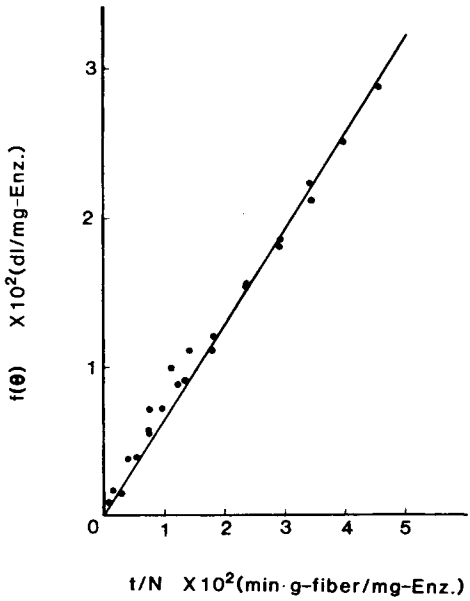

Fig. 5. Relationship between $f(\theta)$ and t/N.

apparent surface is covered, enzymes in bulk solution seems to have to diffuse through small space between twisted filaments for access to vacant sites. In this case, it may take a bit more time for enzymes to be immobilized on active adsorption sites. $f(\theta)$ deviates from the straight line in Fig. 5 in the concentration range over 100 mg enz./dL.

The Comparison of the Calculated Values with Observed Enzyme Concentration

The values of k_a, K_a, and C_0 were substituted into eq. (8), and then the time course of enzyme absorption was calculated. The calculated adsorption curves and observed enzyme concentration are plotted in Figure 6. Comparison of the calculated with found values gives excellent agreement in the initial enzyme concentration range below 100 mg enz./dL. However, in the higher concentration range, there was not good agreement between them. The dotted curves in Figure 7, which indicate calculated results, deviate greatly from experimental values. As free enzymes in the bulk solution have to diffuse through spaces among filaments in order to reach vacant sites, the apparent adsorption rate constant seems to decrease with a progress of adsorption. But, even in the higher concentration range, a linear relationship based on the Langmuir adsorption mechanism exists between $(W/V)\, C_\infty/(C_0 - C_\infty)$ and C_∞ (see Fig. 4). Therefore, $k_a(1 - \alpha\theta)$ was employed for calculation instead of k_a by considering the enzyme diffusion described above (see Appendix). Two real curves in Figure 7 represent the enzyme

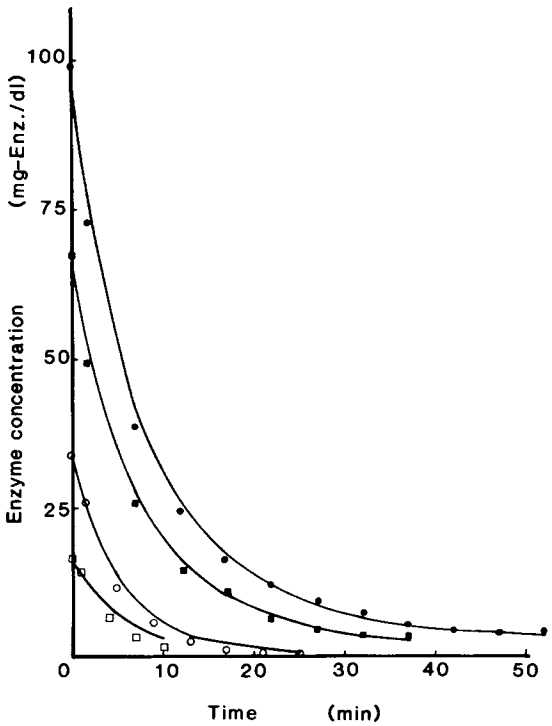

Fig. 6. Comparison of calculated values with observed enzyme concentration C_0 (mg enz./dL): (●) 99; (■) 67; (○) 34; (□) 17.

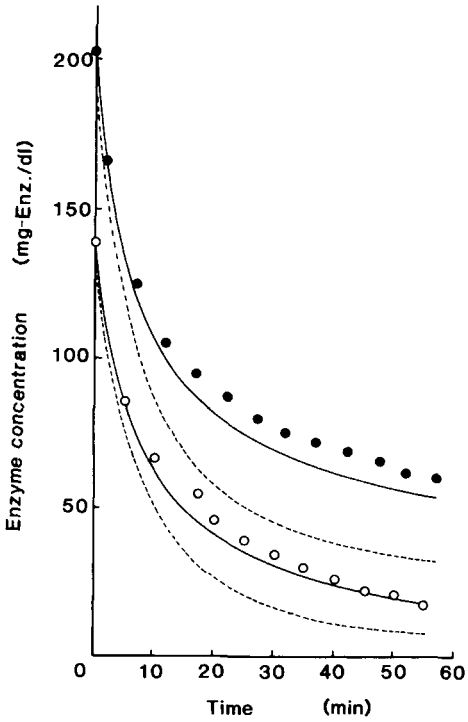

Fig. 7. Comparison of calculated values with observed enzyme concentration C_0 (mg enz./dL): (●) 203; (○) 138.

concentration calculated from eq. (12) in the Appendix at $\alpha = 1$. The deviation between the real curves and observed values is small, and the modification seems to be reasonable.

CONCLUSION

In the initial enzyme concentration range below 100 mg enz./dL, invertase is adsorbed on dimethyl-aminated SFF according to the Langmuir adsorption mechanism.

In the higher concentration range, it was found that the experimental data almost agree with the results calculated with the corrected forward adsorption rate constant.

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APPENDIX

$$-\frac{V}{W}\frac{dC}{dt} = k_a(1-\alpha\theta)C(1-\theta) - \frac{k_a(1-\alpha\theta)}{K_a}\theta \tag{9}$$

Substitution of eq. (2) into eq. (9) gives

$$N\frac{d\theta}{dt} = k_a(-\alpha\theta + 1)\left[\frac{NW}{V}\theta^2 - \left(\frac{NW}{V} + C_0 + \frac{1}{K_a}\right)\theta + C_0\right] \tag{10}$$

$$\text{IC } \theta = 0, \quad \text{at } t = 0$$

Rearranging eq. (10) gives

$$\frac{d\theta}{(-\alpha\theta + 1)[(NW/V)\theta^2 - (NW/V + C_0 + 1/K_a)\theta + C_0]} = \frac{k_a}{N}dt \tag{11}$$

Eq. (11) is integrated to give

$$t = \frac{1}{2(aq^2 - bpq + cp^2)}\left[p \cdot \log\left|\frac{C(p\theta + q)^2}{q^2(a\theta^2 + b\theta + c)}\right| + \frac{(2aq - bp)}{\sqrt{b^2 - 4ac}}\log\left|\frac{(2a\theta + b - \sqrt{b^2 - 4ac})(b + \sqrt{b^2 - 4ac})}{(2a\theta + b + \sqrt{b^2 - 4ac})(b - \sqrt{b^2 - 4ac})}\right|\right]\frac{N}{k_a} \tag{12}$$

in which $a = NW/V$, $b = -(NW/V + C_0 + 1/K_a)$, $c = C_0$, $p = -\alpha$, and $q = 1$.

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