

다른 결정성을 갖는 Cellulose에 대한 Cellulase의 비이온성 계면활성제 용액에서의 흡착거동

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Adsorption Behaviors of Cellulase on Cellulose with Different Crystallinities in Nonionic Surfactant Solution

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ABSTRACT

The adsorption of cellulase on celluloses with different crystallinities was carried out in nonionic surfactant (Tween 20) solution. Highly crystallized celluloses were prepared by enzymatic prehydrolyzation. From the experiments, the Langmuir isotherm parameters, maximum adsorption amount (A_{max}) and adsorption equilibrium constant (K_{ad}) for the adsorption, were obtained in the presence and absence of nonionic surfactant. It was found that the K_{ad} values were decreased by adding Tween 20. This indicates that the adsorption affinity is reduced by nonionic surfactant, and A_{max} decreased with increasing crystallinity under conditions accompanying in both the presence and absence of surfactant. The thermodynamic parameters such as ΔH_a , ΔG_a , and ΔS_a for the adsorption were calculated by using the experimental data. From these results, it was found that the adsorption processes are exothermic reactions in both the presence and absence of surfactant. The heats of adsorption in surfactant solution ($-4.68 \sim -3.62 \text{KJmol}^{-1}$) are smaller than that of the adsorption in the absence of surfactant ($-15.60 \sim -12.10 \text{KJmol}^{-1}$). These results indicated that the tightness of adsorption was reduced by the addition of surfactant. The ΔS_a values were estimated to be positive. This may suggest that the water and solute are released from cellulose on adsorption. The ΔS_a values in surfactant solution are larger than that of the adsorption in the absence of surfactant. This may suggest that the binding of surfactant on hydrophobic region of cellulase cause dispersion of water and solute molecule orienting around the enzyme molecule. The surfactant played an important role in the desorption of enzyme from cellulose functional groups, and enhance the saccharification of the cellulose.

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INTRODUCTION

Cellulosic materials are annually renewable resources. Their Enzymatic hydrolysis has been actively attempted. At present, however, it is difficult to understand perfectly the enzymatic hydrolysis from the theoretical approach and to develop its kinetic even for pure cellulose because of complex and mysterious actions of multicomponent cellulase(1-5). Utilization of the monomeric sugars stored in cellulosic substrates for the production of industrial chemicals and microbial protein requires the previous hydrolysis of the polysaccharides by acid on enzymatic treatment. The steps by which cellulase hydrolyze cellulose are a function of substrate reactivity as well as enzyme activity. Substrate reactivity and enzyme activity must be considered together if an accurate description of cellulose saccharification is to be developed(6).

Cellulose, a linear homopolymer of β -D-glucose, linked by glycosidic bonds, is composed of microfibrils, in which crystalline regions alternate with amorphous regions. The susceptibility of cellulose to enzymatic hydrolysis has been attributed to various structural features of cellulose. These features include crystallinity, molecular structure including conformation, the degree of polymerization, specific surface area and accessibility of the glycosidic linkage(7-11). Of all these, crystallinity of cellulose has usually be suggested to be the major structural feature that influences the hydrolytic degradation by enzyme(7-9).

With these properties of cellulose, the adsorption of cellulase is very important because adsorption is a prerequisite step in the enzymatic hydrolysis reaction of cellulose and detailed studied on the adsorption may lead to better understanding of the mechanism of enzymatic hydrolysis process. And investigation on the adsorption phenomenon has provide much useful information on the action of cellulase in the enzymatic hydrolysis of cellulosic materials. Some important observation on cellulase adsorption have been re-

ported(12-15). These include the effects of various pretreatments of cellulose and sample preparations on the adsorption of cellulase. Nevertheless, the adsorption characteristics of cellulase complex and the mechanism of cellulase adsorption on cellulosic material are not still understood completely. Previous, we have described the adsorption behaviors on microcrystalline cellulose of endo- and exo-type cellulase partially purified from fungus *Trichoderma viride*. It was shown that the maximal synergistic degradation occurs at the specific weight ratio of cellulase components at which the maximal affinity of cellulase components obtains(16). More recent our work have described the adsorption kinetics of exoglucanase in combination with endoglucanase from *Trichoderma viride* on microcrystalline cellulose and its influence on synergistic degradation. It was shown that synergistic degradation of microcrystalline cellulose is dependent on the randomness of the endoglucanase and the tightness and affinity of adsorption(17). Recently, Costanon and Wilke have reported that a surfactant Tween 80 added to the reaction solution increases the rate and extent of saccharification (18). Ooshima et al. suggested that Tween 20 plays an important role in the hydrolysis of crystalline cellulose and that Tween 20 disturbs the adsorption cellulase on cellulose(19,20). Such effects of surfactants may supply more fundamental information on the cellulase adsorption and enzymatic hydrolysis.

In this work, the effect of nonionic surfactant on adsorption of cellulase with different crystallinities was analyzed quantitatively using adsorption kinetic data obtained from the Langmuir isotherm equation, and the action of enzyme in the surfactant solution has been discussed.

MATERIALS AND METHODS

Materials.

The cellulase enzyme, Meicelase TP 60(Lot No.

CEPB-5291), a commercial cellulase preparation of *Trichoderma viride* origin used in this study was kindly provided by Meiji Seika Kaisha Ltd., Tokyo, Japan. Nonionic surfactant as biological detergent (Tween 20) and Sigmacell 100 were obtained from Sigma chemical Co. USA. All other reagents were of analytical grade.

Preparation of prehydrolyzed celluloses.

The H_3PO_4 -treated Sigmacell 100 was prepared using 85% phosphoric acid. The prewetted Sigmacell 100 was mixed with H_3PO_4 solution and was allowed to stand for 1 day at 2°C with cooling. The treated Sigmacell 100 was regenerated or precipitated by adding sodium hydroxide solution. This was washed with distilled water until the wash water was neutral(21). Sigmacell 100 was pretreated with cellulase in 0.1M sodium acetate buffer solution at pH 5.0. 10g of Sigmacell 100 was accurately weighed, immersed in 150ml enzyme buffer solution at pH 5.0, and incubated at 50°C with shaking at 120 strokes/min. The final enzyme concentration was 0.67mg/ml. The reaction was stopped after 12 and 48hr, respectively. The Sigmacell 100 residue was filtered and washed successively with 100ml distilled water, 900ml 1.0M NaCl solution, and 800ml 0.1M sodium acetate buffer solution at pH 5.0. After freeze-drying, this pretreated cellulose was then used as the substrate further for the hydrolysis.

Determination of correlation crystallinity index.

The crystallinities of various cellulosic substrates were measured in this work using a X-ray diffraction analyzer(22). The X-ray diffractogram of various cellulosic substrates showed that they had a different crystallinity index and intensities at the 2θ decreased in parallel with decrease in the crystallinity. The value of the relative crystallinity index(C_c) of each cellulosic substrate was determined for 2θ from 10° to 36° based on the C_c value of 48 hr prehydrolyzed Sigmacell 100 and the H_3PO_4 -treated Sigmacell

100 taken as 1 and 0, respectively. The C_c values of the H_3PO_4 -treated Sigmacell 100, Sigmacell 100, and 12 hr prehydrolyzed Sigmacell 100 were 0, 0.73 and 0.84, respectively, while 48hr pretreated Sigmacell 100 had a C_c value of 1(23). These increase in the correlation crystallinity index indicated that the amorphous portion of Sigmacell 100 was hydrolyzed more quickly than the crystalline portion(24).

Adsorption of cellulase on celluloses with different crystallinities.

Sigmacell 100 and prehydrolyzed cellulose was used as the cellulase adsorbents. A 50 mg cellulose sample was suspended in 5.0ml of 0.1M sodium acetate buffer, pH 4.8 containing 0, and 0.05 wt% Tween 20, and preincubated at a given temperature of 5°C~35°C for 30 min. After preincubation, each 1.0ml of 0.1~2.5mg/ml cellulase was added. The reaction mixture was subjected to reciprocal shaking at 120 strokes/min for 30 min, which is sufficient time to attain the adsorption equilibrium, then centrifuged for 5 min at 5000 rev.min⁻¹. The amount of enzyme in a supernatant was determined by the Lowry method(25) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Adsorption behaviors of cellulase on microcrystalline celluloses.

The adsorption parameter values were determined from the Langmuir adsorption isotherm equation. The adsorption of cellulase can be described as follows(26) :

$$[A] = \frac{[A_{max}] \cdot K_{ad}[E]}{1 + K_{ad}[E]} \quad (1)$$

where A_{max} and K_{ad} are the maximum adsorption amount and adsorption equilibrium constant, respectively. $[E]$ is the concentration of enzyme in liquid phase at adsorption equilibrium. The adsorption isotherm can be rearranged as follows :

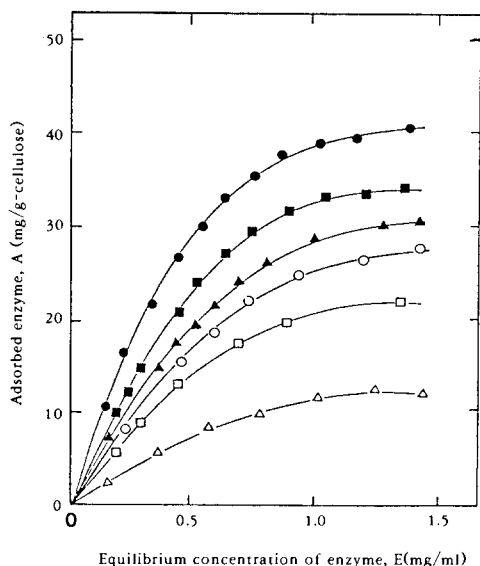


Fig. 1. Adsorption isotherms of cellulase on with different crystallinities of cellulose in the presence and absence of nonionic surfactant at 15°C.

- (1) Surfactant concentration (wt%) 0.00: Sigmacell 100 (●), 12 hr-prehydrolyzed Sigmacell 100 cellulose (■), 48 hr-prehydrolyzed Sigmacell 100 cellulose (▲).
- (2) Surfactant concentration (wt%) 0.05: Sigmacell 100 (○), 12 hr-prehydrolyzed Sigmacell 100 cellulose (□), 48 hr-prehydrolyzed Sigmacell 100 cellulose (△).

$$\frac{[E]}{[A]} = \frac{1}{K_{ad} \cdot [A_{max}]} + \frac{1}{[A_{max}]} \cdot [E] \quad (2)$$

Adsorption equilibrium constant (K_{ad}) and maximum adsorption amount (A_{max}) were determined from the plots of $[E]/A$ vs. $[E]$, respectively. Fig. 1 shows that the adsorption isotherms of cellulase at 15°C on celluloses having different crystallinity in the presence and absence of nonionic surfactant was varied with the crystallinity of cellulose. Then the plots of $[E]/[A]$ vs. $[E]$ gave fairly good straight lines as shown

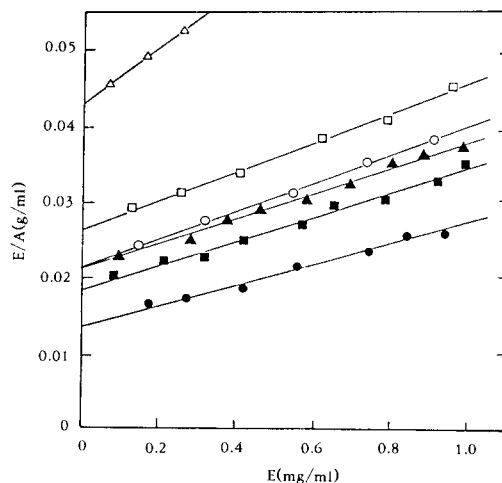


Fig. 2. Langmuir plots for the adsorption isotherms of cellulase on with different crystallinities of cellulose in the presence and absence of nonionic surfactant at 15°C.

- (1) Surfactant concentration (wt%) 0.00: Sigmacell 100 (●), 12 hr-prehydrolyzed Sigmacell 100 cellulose (■), 48 hr-prehydrolyzed Sigmacell 100 cellulose (▲).
- (2) Surfactant concentration (wt%) 0.05: Sigmacell 100 (○), 12 hr-prehydrolyzed Sigmacell 100 cellulose (□), 48 hr-prehydrolyzed Sigmacell 100 cellulose (△).

in Fig. 2. These results imply that the adsorption of cellulase on cellulose having different crystallinity in the presence and absence of nonionic surfactant fits the Langmuir isotherm calculated adsorption parameter values as shown in Table 1. The K_{ad} value is an intensive property of adsorption and is a measurement for the adsorption affinity. K_{ad} values decrease with an increase in the crystallinity. The K_{ad} values in the presence or absence of surfactant depended on temperature, i.e., K_{ad} values decreased with increasing temperature, and K_{ad} values in the presence of surfactant with the same substrate are smaller than that in its absence. It indicates that

Table 1. Langmuir parameters in adsorption on cellulose

Temp. (°C)	Surfactant Conc. (wt%) 0.00						Surfactant Conc. (wt%) 0.05					
	A		B		C		A*		B*		C*	
	A_{max}	$K_{ad} \times 10^{-4}$	A_{max}	$K_{ad} \times 10^{-4}$	A_{max}	$K_{ad} \times 10^{-4}$	A_{max}	$K_{ad} \times 10^{-4}$	A_{max}	$K_{ad} \times 10^{-4}$	A_{max}	$K_{ad} \times 10^{-4}$
5	68.25	9.69	65.47	6.42	64.24	5.21	57.97	4.11	55.64	3.92	24.01	3.60
15	66.24	7.92	63.49	5.29	63.08	4.05	55.91	4.04	53.60	3.67	23.32	3.45
25	65.17	6.55	61.00	4.50	60.14	3.54	53.29	3.62	47.26	3.54	22.79	3.30
35	64.72	4.96	59.22	3.25	56.42	3.10	52.00	3.42	45.20	3.20	21.74	3.08

A, A* ; Sigmacell 100

B, B* ; 12hr-prehydrolyzed cellulose, Sigmacell 100

C, C* ; 48hr-prehydrolyzed cellulose, Sigmacell 100

the adsorption affinity is reduced by nonionic surfactant. Therefore, these results mean that cellulase is desorbed by surfactant. The A_{max} is an extensive one, which is proportional to the number of adsorption sites per unit surface and the enzyme accessible specific surface area of adsorbent(17). The specific surface area is probably expected to decrease as crystallinity of the cellulose increase, because the crystalline portion of cellulose has smaller specific surface area. In our experimental data, A_{max} decreased with an increase in crystallinity under conditions accompanying both the presence and absence of surfactant. The A_{max} values in the presence of surfactant are smaller than that in its absence. It appears that surfactant will aid enzyme, which is tightly adsorbed to the substrate surface, to desorb from the substrate.

Thermodynamic properties of cellulase adsorption.

Table 1 shows the dependency of Langmuir parameters at various temperatures which were estimated from the data in Fig. 2. The thermodynamic parameters in the adsorption of cellulase can be evaluated from the K_{ad} values. One of the thermodynamic parameters was obtained from the van't Hoff equation :

$$\ln K_{ad} = -\frac{\Delta H_a}{RT} \quad (3)$$

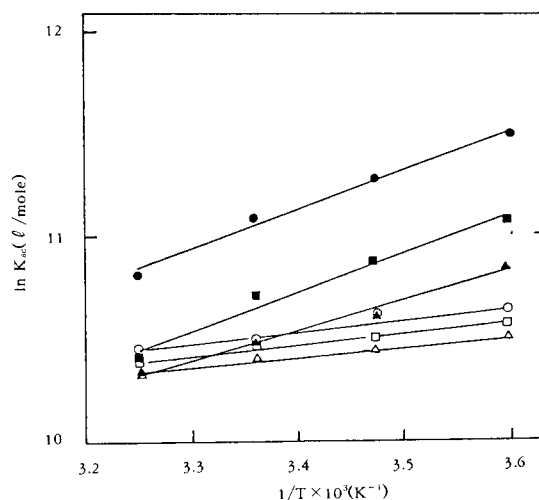


Fig. 3. Temperature dependency of adsorption equilibrium constant(K_{ad}).

- (1) Surfactant concentration(wt%) 0.00: Sigmacell 100 (●), 12 hr-prehydrolyzed Sigmacell 100 cellulose(■), 48 hr-prehydrolyzed Sigmacell 100 cellulose (▲).
- (2) Surfactant concentration(wt%) 0.05: Sigmacell 100 (○), 12 hr-prehydrolyzed Sigmacell 100 cellulose(□), 48 hr-prehydrolyzed Sigmacell 100 cellulose (△).

where ΔH_a is the value of enthalpy of adsorption, and R is the gas constant. Equation(3) shows that a plot of $\ln K_{ad}$ against $1/T$ has a slope equal to $\Delta H_a/R$ as shown in Fig. 3. The value of K_{ad} can be

Table 2. Thermodynamic parameters in adsorption on cellulose at 15 °C

Parameters	Surfactant Conc. (wt %) 0.00			Surfactant Conc. (wt %) 0.05		
	A	B	C	A*	B*	C*
ΔH_a (KJ/mol)	-15.60	-15.51	-12.10	-4.68	-4.54	-3.62
ΔG_a (KJ/mol)	-27.00	-26.04	-25.42	-25.40	-25.17	-25.02
ΔS_a (JK/mol)	41.67	36.57	46.25	71.95	67.43	78.82

A, A* ; Sigmacell 100

B, B* ; 12hr-prehydrolyzed cellulose, Sigmacell 100

C, C* ; 48hr-prehydrolyzed cellulose, Sigmacell 100

used to obtain the value of free energy of adsorption (ΔG_a):

$$\Delta G_a = -RT \ln K_{ad} \quad (4)$$

The value of ΔG_a is used with the value of ΔH_a to obtain the value of entropy of adsorption (ΔS_a):

$$\Delta S_a = \frac{\Delta H_a - \Delta G_a}{T} \quad (5)$$

Table 2 shows the values of enthalpy, free energy, and entropy accompanying the adsorption which were obtained with Eqs. (3), (4), and (5), respectively. The average molecular weight of cellulase required to obtain the thermodynamic parameters was 60,000(14). It was found from the negative values of ΔH_a that the adsorption reaction was an exothermic and adsorption enthalpy-controlled reaction. Namely, the enthalpy term plays the principal role in the adsorption in the temperature range. The heats of adsorption decreased with an increase in crystallinity under conditions accompanying both the presence and absence of surfactant. Also, the heats of adsorption in the surfactant solution ($-4.68 \sim -3.62 \text{KJmol}^{-1}$) are smaller than that of the adsorption in the absence of surfactant ($-15.60 \sim -12.10 \text{KJmol}^{-1}$). Kim et al.(17) described the tightness of adsorption as changes of adsorption enthalpy. Therefore, these results indicated that the tightness of adsorption was reduced by the

addition of the surfactant. This indicates that it may be related to surfactant binding on a cellulase. The cellulose and cellulase were known to have hydrophobic regions(27). Since the tightness of adsorption are weakened by the presence of nonionic surfactant, it is thought that the hydrophobic interaction between cellulase and cellulose by surfactant help to separate the cellulase. The large negative values of ΔG_a indicate that the adsorption of different crystallinities of the cellulose in the presence or absence of nonionic surfactant take place spontaneously. The ΔS_a values were estimates to be positive. This may suggest that the water and solute are released from cellulose on the adsorption. The ΔS_a values in surfactant solution are larger than that of the adsorption in the absence of surfactant. Perhaps this may suggest that the binding of surfactant on hydrophobic region of cellulase cause dispersion of water and solute molecule orienting around the enzyme molecule. From these facts, it was found that the surfactant enhance the enzyme desorption from insoluble substrates. It was thought that enzyme, which adsorbed first to the cellulose functional group, did not desorb, thus prevention further reaction with the remaining free enzyme. In this case, therefore, surfactant may play an important role in the desorption of the enzyme from cellulose functional groups, and enhance the saccharification.

요 약

비이온성 계면활성제(Tween 20)용액에서 결정성이 다른 셀룰로오스에 대한 셀룰라아제의 흡착을 알아 보았다. 결정성이 큰 셀룰로오스는 효소적 가수분해에 의한 전처리로부터 얻었다. 실험을 통하여, 흡착에 대한 Langmuir 등온파라미터로서 최대흡착량(A_{max}) 및 흡착평형상수(K_{ad})를 얻었다. 이 때 각 K_{ad} 의 값들은 Tween 20의 첨가에 의해 감소하였다. 이것은 흡착 친화력이 계면활성제에 의하여 감소되어짐을 나타내 준다. A_{max} 값은 셀룰로오스의 결정성이 증가할수록 감소하였으며, 계면활성제 첨가시 더욱 감소되었다. 또한 흡착에 대한 열역학적 파라미터, ΔH_{ad} , ΔG_{ad} , 및 ΔS_{ad} 가 위에서 얻어진 값들로부터 결정되었다. 이러한 결과로부터 모든 흡착과정이 발열반응임을 알 수 있었다. 흡착 엔탈피값은 계면활성제 존재 하에서 감소되어 지는데, 이것은 흡착의 견고함이 계면활성제의 첨가로 셀룰로오스와 셀룰라아제 사이의 hydrophobic interaction 때문에 약해짐을 알려 준다. 흡착 엔트로피값은 모두 양의 값을 보여 주는데, 이것은 셀룰로오스에 셀룰라아제가 흡착할 때 물이나 용질이 빠져나가기 때문으로 생각된다. 또한, 계면활성제 첨가시 엔트로피가 더욱 증가하는 것은, 계면활성제가 셀룰로오스나 셀룰라아제에 흡착함에 따라 더 많은 물이나 용질이 빠져나가기 때문으로 생각된다. 비이온성 계면활성제는 셀룰로오스 표면의 실활(inactivation)된 셀룰라아제의 탈착을 도와 가수분해를 향상시키는 것으로 보여진다.

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